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Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys dentatus*)

Andre Buchheister and Robert J. Latour

Abstract: Migratory and mobile fishes such as summer flounder (*Paralichthys dentatus*) often utilize dietary resources with stable isotope signatures that vary over time and space, potentially confounding diet analyses if tissues with slow turnover are sampled before reaching isotopic equilibrium. A laboratory diet-shift study was conducted using juvenile and young adult summer flounder to (*i*) determine isotopic turnover rates and fractionations of $\delta^{13}C$ and $\delta^{15}N$ in liver, whole blood, and white muscle and (*ii*) estimate the relative importance of growth and metabolic processes on isotopic turnover. Isotopic turnover rates were consistently ranked liver > blood > muscle owing to increased metabolic activities of liver and blood. Carbon and nitrogen half-lives ranged from 10 to 20 days (liver), 22 to 44 days (blood), and 49 to 107 days (muscle), indicating that liver and blood are more useful than muscle as shorter-term dietary indicators for summer flounder and other migratory fishes. Growth-based fractionation estimates of wild flounder tissues ranged from 0.71‰ to 3.27‰ for carbon and from 2.28‰ to 2.80‰ for nitrogen and included the first explicit estimates for isotopic fractionation in fish blood. A generalized model for predicting the time scale of isotopic turnover from growth-based turnover parameters was also developed to help evaluate isotopic equilibrium assumptions of fishes in the field.

Résumé : Les poissons migrateurs et mobiles, tels que le cardeau d'été (*Paralichthys dentatus*), utilisent souvent des ressources alimentaires dont les signatures d'isotopes stables varient dans le temps et l'espace, ce qui peut fausser les analyses de régime alimentaire si on échantillonne des tissus à faible taux de renouvellement avant qu'ils n'aient atteint l'équilibre isotopique. Nous avons procédé à une étude de changement de régime en laboratoire chez des juvéniles et des jeunes adultes du cardeau d'été afin (*i*) de déterminer les taux de renouvellement des isotopes et le fractionnement de $\delta^{13}C$ et de $\delta^{15}N$ dans le foie, le sang entier et le muscle blanc et (*ii*) d'estimer l'importance relative de la croissance et des taux métaboliques dans le renouvellement des isotopes. À cause des activités métaboliques plus importantes du foie et du sang, les taux de renouvellement isotopique suivent généralement l'ordre foie > sang > muscle. Les demi-vies du carbone et de l'azote varient de 10–20 jours (foie), à 22–44 jours (sang) et à 49–107 jours (muscle), ce qui indique que le foie et le sang sont de meilleurs indicateurs alimentaires à court terme que le muscle chez le cardeau d'été et les autres poissons migrateurs. Les estimations du fractionnement relié à la croissance dans les tissus de cardeaux sauvages varient de 0,71–3,27 ‰ pour le carbone et de 2,28–2,80 ‰ pour l'azote et incluent les premières estimations explicites du fractionnement isotopique dans le sang de poisson. Nous mettons au point un modèle généralisé pour prédire le taux de renouvellement isotopique pour les variables du renouvellement reliées à la croissance afin d'aider à évaluer les présuppositions concernant l'équilibre isotopique des poissons en nature.

[Traduit par la Rédaction]

Introduction

Stable isotope analysis is becoming an increasingly more common tool for elucidating trophic interactions and energy pathways in food webs (Fry 2006). Stable isotopes act as dietary tracers and can be used to calculate the assimilated contributions of different prey sources to a consumer (Phillips and Gregg 2003; Fry 2006). Dietary calculations and many general conclusions from stable isotope studies are often based on two assumptions: (*i*) sampled tissues are in equilibrium with the sampled diet, and (*ii*) fractionations between the consumed food and the assimilated tissue are known (Post 2002). However, these critical assumptions are often not validated for individual field studies.

The assumption that a consumer is equilibrated isotopically to the sampled diet can be violated easily for many mobile or migratory fish species. Attainment of isotopic equilibrium depends on an animal's isotopic turnover rate and its dietary consistency. Isotopic turnover rate refers to how quickly the isotopic signature in a body tissue changes

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following a dietary change. Proper consideration of turnover rates becomes crucial in assessing diets of the many species that migrate between different coastal, estuarine, or freshwater habitats (e.g., Murdy et al. 1997; Carpenter 2002), because they move between environments with potentially variable isotopic characteristics, rely on seasonally abundant prey types, and generally exhibit ontogenetic shifts in feeding. Aside from increasing the sampling effort needed to adequately characterize prey items (a critical component of isotopic mixing models), dietary changes can prevent a predator from equilibrating isotopically to their prey because isotopic signatures of prey usually vary by species. In addition, the isotopic values of a single prey species can vary over space and time (Perga and Gerdeaux 2005), hindering equilibrium. Dietary inconsistencies (both taxonomic and isotopic) therefore become particularly problematic for migratory species and for species with slow turnover rates, potentially compromising the validity of any conclusions based on stable isotope analyses.

Increasing the temporal resolution of isotopic dietary analyses and minimizing violations of the isotopic equilibrium assumption can be achieved through examination of body tissues with faster turnover rates. Isotopic turnover is mediated by two general processes: the dilution of existing mass by new mass synthesized from recently consumed prey (i.e., growth), and the replacement or conversion of existing tissue using material synthesized from recent diet (i.e., metabolism) (Hesslein et al. 1993). Consequently, tissues that are more metabolically active tend to respond more quickly to dietary changes (Tieszen et al. 1983; Hobson and Clark 1992*a*, 1992*b*), whereas other tissues such as muscle can require over one year to equilibrate to a constant diet (Hesslein et al. 1993; MacAvoy et al. 2001). Although the number of laboratory studies examining turnover rates in fish tissues has increased over the last 10 years (e.g., MacAvoy et al. 2001; MacNeil et al. 2006), many tissues remain understudied relative to muscle. Tissue-specific turnover rates can allow researchers to tailor the sampled tissue to an appropriate time scale for the study's objective. For example, tissues with higher isotopic turnover rates can address prey utilization on shorter temporal scales (Perga and Gerdeaux 2005) or reflect the nutritional contribution of seasonally available migratory prey (MacAvoy et al. 2001). Use of faster turnover tissues may thus help clarify the interpretation of isotopic data for mobile and migratory species experiencing high spatial or temporal heterogeneity in prey isotope signatures.

Calculation of diets from stable isotope ratios of multiple tissues requires appropriate fractionation values for each tissue and isotope. Fractionation refers to the process in which ratios of stable isotopes change between prey and consumer during metabolic processing of consumed material (Fry and Sherr 1984; Fry 2006). Studies often assume fractionations of ~3.4‰ for nitrogen and 0‰–1‰ for carbon stable isotopes (Minagawa and Wada 1984; Post 2002; Sweeting et al. 2007); however, these values are based on muscle or whole-body samples. Fractionation values can vary among tissues (Hobson and Clark 1992*b*) and species (Tieszen et al.1983; Vander Zanden and Rasmussen 2001), but empirical measurements of' fractionation values for fish tissues other than muscle are lacking in the literature. To our knowledge, no explicit measurements of fractionation have been published to date for fish blood, and few exist for fish liver (Trueman et al. 2005; Logan et al. 2006; Sweeting et al. 2007). Use of inaccurate fractionations can introduce substantial errors into diet estimates, particularly when the isotopic separation among prey sources is small (Vander Zanden and Rasmussen 2001). Therefore, as isotopic studies become more common and utilize a greater variety of fish tissues, it is crucial to have robust and valid measures of fractionation for the tissue of interest.

In this study, a controlled diet-shift experiment was conducted using summer flounder (*Paralichthys dentatus*), a migratory species that utilizes estuarine and coastal habitats along the eastern seaboard of North America. The objectives were to (*i*) empirically determine the turnover rates and fractionations of carbon (C) and nitrogen (N) stable isotopes in liver, whole blood, and muscle of juvenile and young adult summer flounder, and (*ii*) model the relative contributions of metabolism and growth to the process of isotopic turnover in each tissue. Results from this study facilitate more accurate application of stable isotope techniques to quantify diet and productivity of summer flounder and other migratory fishes by providing critical fractionation values and describing the dietary time scale represented by different body tissues. This study also demonstrates the generic need for more baseline information on tissue-specific turnover rates and fractionations to aid in isotopic studies of fishes, particularly those that are seasonally estuarine-dependent.

Materials and methods

Fish rearing

A group of juvenile, age-0 summer flounder (n = 72) was purchased from a commercial fish hatchery (GreatBay Aquaculture, Portsmouth, New Hampshire). These fish had been spawned from wild broodstock and raised on a similar diet of formulated feed. Hatchery fish were transported from New Hampshire to the Virginia Institute of Marine Science (VIMS) in Gloucester Point, Virginia, and allowed to acclimate to an experimental tank for 3 weeks while maintaining the previous diet. A second group of age-0 juveniles (n = 55) captured from the lower Chesapeake Bay during August and September of 2007 using a bottom trawl was maintained in captivity for 2–3 months on a constant diet of squid. The squid diet mimicked the isotopic signature of food items regularly consumed by juvenile summer flounder in Chesapeake Bay (A. Buchheister, VIMS, unpublished data) and thus maintained the wild fish at roughly the same isotopic values as at initial capture. The hatchery (H) and wild (W) fish were treated separately throughout the experiment because of the differences in their rearing histories and initial isotopic values. The H and W groups of fish were thus maintained separately in 450 gallon recirculating tanks with water filtered to 1 µm obtained from the York River, a tributary of the Chesapeake Bay. Average (± standard deviation, SD) tank temperatures (W group, 19.9 ± 0.7 °C; H group, 20.4 ± 0.7 °C) and salinities (26.1 ± 0.9 ppt) represented intermediate values found within bottom waters of Chesapeake Bay from May to November (Chesapeake Bay Program 2007). Fish were kept on a 13 h light – 11 h dark schedule.

Two days prior to the start of the diet-shift experiment in early November of 2007, all individuals were marked with unique coded wire tags (Northwest Marine Technology, Shaw Island, Washington) inserted into the blind-side musculature and measured for total length (mm) and weight (mg) (Table 1). The minute tags did not appear to affect fish behavior, and a minimal proportion of fish (2.6%) did not retain their tags (likely due to insertion error), making the coded wire tags ideal for the study. At the start of the experiment, seven fish were sacrificed from each experimental group to obtain baseline isotopic signatures for muscle, liver, and blood. The remaining fish were all switched to a constant diet of commercially purchased krill, *Euphausia superba* (Fishalicious Foods, Custer, Washington), which had substantially different isotopic signatures from both summer flounder groups and their previous diets. Fish were fed up to 5% of their body weight per day in an attempt to replicate realistic growth rates of fish in the wild (Rountree and Able 1992).

To monitor turnover of stable isotopes, four to six individuals from each group were randomly sampled and euthanized at specified time periods. Towards the end of the experiment, up to 10 individuals were sampled to use all animals. All protocols pertaining to sampling and euthanizing of experimental fish were approved by the College of William and Mary's Institutional Animal Care and Use Committee. Fish were sampled at 7, 13, 24, 35, 49, 67, 89, 126, 155, and 180 days from the start of the experiment. Three samples of the krill diet were periodically sampled to assure consistency in the dietary isotopic signature.

Processing of sampled fish

Summer flounder selected for isotopic determination were removed from the water at least 12 h after the previous feeding and measured for total length (mm) and weight (mg). Samples of whole blood (0.5–1 mL) were extracted from the caudal vein using a 25-gauge needle and sterile syringe and then stored in a precombusted glass vial. Fish were then placed in Ziploc bags (S.C. Johnson & Son Inc., Racine, Wisconsin) and euthanized in an ice-water bath. In the laboratory, samples of liver and dorsal muscle (above the pectoral fin on the ocular side) were excised, rinsed with deionized water, and placed in precombusted glass vials. Coded wire tags were recovered from the musculature of each fish to identify individuals. Tissue samples were dried (45 °C for at least 2 days) and ground and then a 0.8–1.2 mg subsample was packaged into a 4 mm × 6 mm tin capsule for stable isotope analysis.

Prepared samples were analyzed at the University of California – Davis Stable Isotope Facility (Davis, California) using a continuous flow isotope ratio mass spectrometer (Europa Hydra 20/20; Europa Scientific, Cambridge, UK). Stable isotope ratios are reported in relation to conventional standards:

$$(1) \qquad \delta X = \frac{(R_{\mathrm{sample}} - R_{\mathrm{standard}})}{R_{\mathrm{standard}}} \times 1000$$

where X is the stable isotope of C or N, and R is the mass ratio of the heavy to light stable isotope (e.g., $^{15}N/^{14}N$) for either the sample or the standard. The conventional standards used for the analyses were Pee Dee Belmnite for C and air for N, and δX is reported in per mil (‰). Repeated measurements of a calibration standard indicated that instrument precision (SD) was 0.15‰ and 0.08‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Ratios of C to N (C:N) were calculated from measured amounts of C and N in each sample.

Lipid correction for livers

A mathematical correction was used to normalize liver $\delta^{13}C$ values due to the high lipid concentrations in this tissue that bias $\delta^{13}C$ (DeNiro and Epstein 1977; Logan et al. 2008). A subsample of livers was used to characterize the relationship between raw C:N and the bias in $\delta^{13}C$ due to lipids by measuring the isotopic value before ($\delta^{13}C_{NE}$) and after ($\delta^{13}C_{LE}$) lipid extraction (Appendix A). Of a suite of models, the model by McConnaughey and McRoy (1979) best represented the data (Appendix A), and consequently the lipid bias of $\delta^{13}C$ was modeled as

$$(2) \qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = D\left(\theta + \frac{3.90}{1 + 287/L}\right)$$

$$\text{where} \quad L = \frac{93}{1 + (0.246 \times C{:}N - 0.775)^{-1}}$$

In eq. 2, L represents the sample lipid content as a function of C:N, and D represents protein–lipid discrimination. The parameters D and θ were estimated (± standard error, SE) as $D = 6.2883 \pm 1.3519$ and $\theta = 0.0612 \pm 0.1527$. Based on measured C:N, eq. 2 was used to adjust all raw liver $\delta^{13}C$ to values corresponding to a lipid-extracted $\delta^{13}C$. Lipid corrections were not applied to muscle and blood $\delta^{13}C$ values because of relatively low C:N values and limited variability across individuals (mean C:N ± SD: muscle, 3.40 ± 0.13; blood, 4.12 ± 0.39; liver, 14.54 ± 4.68), which suggests that any lipid effects on $\delta^{13}C$ would be negligible in these tissues (Kiljunen et al. 2006).

Growth-based modeling

Changes in stable isotope ratios were modeled as a function of relative growth since the diet switch. The growth-based model predicts the isotopic value of a fish as

$$(3) \qquad \delta_{W_R} = \delta_f + (\delta_i - \delta_f)W_R^{\,c}$$

(Fry and Arnold 1982), where δ_f is the expected isotopic value when completely equilibrated to the new diet and δ_i is the initial isotopic signature prior to the diet shift. The average isotopic value for the seven fish sacrificed before the diet shift was used as the estimate for δ_i in the model. The relative increase in weight of each fish (W_R) was calculated as the final wet weight divided by the initial wet weight. The variable δ_{W_R} is the measured isotopic value for a fish given its increase in weight, and c is a turnover rate constant. Both δ_f and c were estimated using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina). In this model, if $c = -1$, then growth is entirely responsible for turnover, whereas if $c < -1$, metabolism is contributing to turnover of the stable isotope in the tissue, with more negative values representing greater contributions by metabolism (Fry and Arnold 1982). A t test was used to test c against a null hypothesis of $c = -1$ (Zar 1999).

Fractionations of $\delta^{15}N$ and $\delta^{13}C$ between the diet and each tissue (Δ_{TISSUE}) were calculated as

Table 1. Initial total length and wet weight of hatchery (H) and wild (W) summer flounder (*Paralichthys dentatus*) sampled for stable isotope analysis.

		Total length (mm)				Wet weight (g)			
Group	*n*	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
H	66	176	19	130	230	58.8	18.3	26.3	124.9
W	48	215	25	131	255	102.0	33.2	29.3	185.3

Note: SD, standard deviation; min., minimum; max, maximum.

$$(4)\qquad \Delta_{\mathrm{TISSUE}} = \delta_f - \delta_d$$

(Minagawa and Wada 1984), where δ_d is the mean stable isotope signature for samples of krill diet. Standard errors for the fractionation estimate were based on errors associated with the model estimate of δ_f (SE_{δ_f}) and the standard error of the mean for δ_d (SE_{δ_d}):

$$(5)\qquad \mathrm{SE}_{\Delta_{\mathrm{TISSUE}}} = \sqrt{\mathrm{SE}_{\delta_f}{}^2 + \mathrm{SE}_{\delta_d}{}^2}$$

The amount of relative growth needed to achieve an α percent turnover of δ^{13}C or δ^{15}N ($G_{\alpha/100}$) was calculated as

$$(6)\qquad G_{\alpha/100} = \exp\left(\frac{\ln(1-\alpha/100)}{c}\right)$$

The growth-based half-life ($G_{0.5}$) is obtained when α = 50% and represents the amount of growth needed for a 50% conversion between the initial and final isotopic values. The fractions of new tissue derived from growth (D_g) and from metabolism (D_m) were calculated at the midpoint between the old and new isotopic values (Witting et al. 2004):

$$(7)\qquad D_g = \frac{2(G_{0.5}-1)}{G_{0.5}}$$

$$(8)\qquad D_m = \frac{(2-G_{0.5})}{G_{0.5}}$$

However, note that the equations originally published by Witting et al. (2004) were misprinted (D.A. Witting, NOAA Fisheries Restoration Center, 501 West Ocean Boulevard, Long Beach, CA 90802, USA, personal communication, 2008).

Time-based modeling

Changes in stable isotope ratios were also modeled as a function of time since the diet switch. The time-based exponential model (Hesslein et al. 1993) describes the isotope value of a fish at time t (δ_t) as

$$(9)\qquad \delta_t = \delta_f + (\delta_i - \delta_f)e^{-(k'+m)t}$$

where δ_f and δ_i are as previously defined and m is the metabolic turnover constant. The group-specific growth rate constant, k', was estimated by fitting an exponential growth model to the available data:

$$(10)\qquad W_f = W_i e^{k't}$$

(Ricker 1979), where W_i is a fish's initial wet weight and W_f is the final weight when sampled on day t. Because of different initial sizes and different growth rates between groups, the k' parameter was estimated separately for the wild and hatchery groups using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina). In eq. 9, if turnover is due to growth alone, then m = 0. The values of δ_t, δ_i, and t were measured or calculated, and δ_f and m were estimated using nonlinear regression.

Diet–tissue fractionations (±SE) were also derived from time-based model estimates of δ_f using eqs. 4 and 5. The length of time needed to achieve an α percent turnover of δ^{13}C or δ^{15}N was calculated as

$$(11)\qquad T_{\alpha/100} = \frac{\ln(1-\alpha/100)}{-(k'+m)}$$

(Tieszen et al. 1983). Half-life ($T_{0.5}$) is obtained when α = 50%.

The relative contributions of growth (k') and metabolism (m) were calculated as the ratio of each parameter to the sum of the two parameters. This calculation yields the proportion of turnover attributable to growth (P_g) and to metabolism (P_m). A t test was used to test m against a null hypothesis of m = 0 (Zar 1999). To assess growth patterns of the wild and hatchery populations over the duration of the experiment, specific growth rates (k) were calculated for each individual using the exponential growth model, eq. 10, by substituting k for k' (Ricker 1979).

Model fitting and selection

An information-theoretic approach to model selection was used to assess the best parameterizations for the growth- and time-based models. Given the use of two experimental groups (hatchery and wild fish), we were interested in determining whether the groups were best parameterized separately (i.e., group-specific c or m, or group-specific δ_f), or whether the data could be pooled and a single parameter estimate deemed valid for both groups. Additionally, models could be parameterized to either include or exclude a metabolic contribution to turnover to examine the relative importance of metabolism on the turnover process in each tissue. Six potential models were generated as follows: model A, group-specific turnover parameter and group-specific δ_f; model B, group-specific turnover and a single pooled δ_f estimate; model C, single turnover parameter and group-specific δ_f; model D, single turnover parameter and a single δ_f; model E, no metabolic contribution to turnover (i.e., c = –1 in eq. 3 or m = 0 in eq. 9) and group-specific δ_f; and lastly model F, no metabolic contribution to turnover and a single δ_f estimate. These six model formulations were fitted to each isotope–tissue combination and assessed separately for the growth- and time-based methods.

Objective evaluations of the best model parameterization were based on Akaike's information criterion corrected for small sample sizes (AIC_c) calculated for each model as

$$(12)\qquad \mathrm{AIC_c} = n\left(1 + \ln\left(\frac{2\pi \times \mathrm{RSS}}{n}\right)\right) + 2K + \frac{2K(K+1)}{n-K-1}$$

where n is the sample size, RSS is the residual sum of squares calculated using nonlinear regression (SAS version 9.2; SAS Institute Inc., Cary, North Carolina), and K is the number of estimable parameters in the model (Burnham and Anderson 2002; Kimura 2008). $\mathrm{AIC_c}$ differences ($\Delta\mathrm{AIC_c}$) among the six models were calculated as

$$(13)\qquad \Delta\mathrm{AIC_c} = \mathrm{AIC}_i - \mathrm{AIC_{min}}$$

where AIC_i is the $\mathrm{AIC_c}$ of model i, and $\mathrm{AIC_{min}}$ is the lowest $\mathrm{AIC_c}$ of the competing models. The model with the most empirical support generates a $\Delta\mathrm{AIC_c} = 0$, and we only present parameter estimates for the best of the six models. As a general rule of thumb, $\Delta\mathrm{AIC_c}$ values from 0 to 2 indicate substantial support for the model, $\Delta\mathrm{AIC_c}$ values from 4 to 7 suggest considerably less support, and $\Delta\mathrm{AIC_c}$ values >10 indicate essentially no support for that model (Burnham and Anderson 2002).

For each isotope and tissue, regression assumptions of normality were supported by histograms of the residuals and by Kolmogorov–Smirnoff tests conducted at different levels of the independent variable. Levene's tests and residual plots indicated that homogeneity of variance was not supported for the growth- and time-based models of δ^{15}N for muscle and liver or for δ^{13}C turnover in liver. These models were weighted by the inverse of the variance of the response variable to account for the heteroscedasticity in the estimation and fitting procedures (Neter et al. 1990). Because of heteroscedasticity, eq. 10 was also weighted using the inverse of the W_f variance (calculated at each time period) as the weighting factor (Neter et al. 1990).

Results

Growth

Summer flounder grew well, reaching lengths > 300 mm and weights > 375 g during the 180-day experiment (maximum length and weight of 325 mm and 446.0 g, respectively). Several hatchery individuals more than quadrupled in weight (Fig. 1). Some individuals died after jumping out of the tanks, but a total of 66 hatchery fish and 51 wild fish were sampled during the experiment. Most fish sampled after 60 days of the experiment appeared to have reached sexual maturity based on macroscopic staging; however, no fish were reproductively active.

Specific growth rates (k) varied across individuals, across time, and between groups (Fig. 2). Overall, individual growth rates ranged from –0.010 to 0.024 day^{-1}, but only three fish experienced negative growth rates, or a loss of weight. Hatchery fish tended to grow more rapidly than wild fish, exhibiting the highest k values, particularly in the first 50 days of the experiment. Group-specific growth rates (k') reflected this difference between groups with k' values (±SE) of $8.16 \times 10^{-3} \pm 1.65 \times 10^{-4}$ day^{-1} (H group) and $6.51 \times 10^{-3} \pm 2.51 \times 10^{-4}$ day^{-1} (W group). Growth rates increased initially in both groups before leveling off and then decreasing slightly towards the end of the experiment. The decrease in growth rates was particularly evident in the W_R trends of wild fish over time (Fig. 1). Declines in individual growth rates corresponded with reduced consumption rates that were observed during the second half of the experiment, particularly for the wild fish.

Fig. 1. Relative growth (W_R) for individual summer flounder (*Paralichthys dentatus*) from both hatchery (open triangles) and wild (solid circles) groups. Estimated growth trajectories for each group were based on group-specific growth rates, k' (hatchery group, broken line; wild group, continuous line).

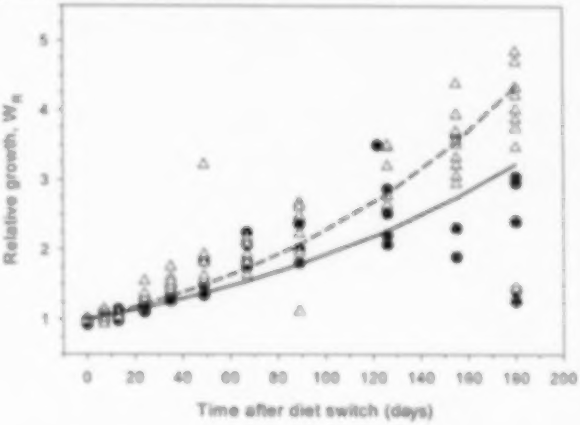
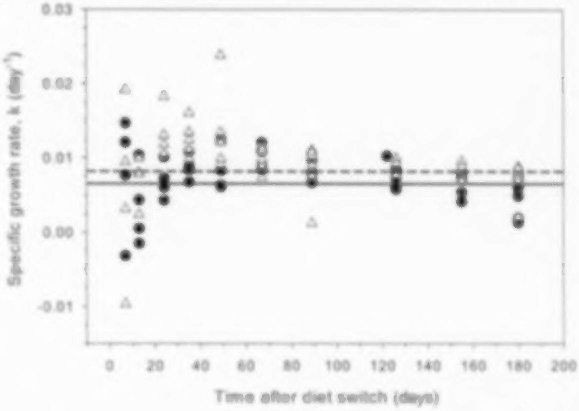


Fig. 2. Specific growth rates (k) calculated for individual summer flounder (*Paralichthys dentatus*) for both hatchery (open triangles) and wild (solid circles) groups. Estimated group-specific growth rates (k') for hatchery (8.16×10^{-3} day^{-1}) and wild (6.51×10^{-3} day^{-1}) groups are represented by broken and continuous lines, respectively.



Model selection

The best model parameterization (models A–F) varied for each method (growth- vs. time-based), isotope, and tissue combination. For growth-based modeling, liver and blood turnover were best described with separate c and δ_f parameters for hatchery and wild groups (model A; Table 2). Muscle δ^{13}C and δ^{15}N turnover was better parameterized with a single δ_f estimated for both groups; however, for the δ^{13}C model, c was estimated for each group individually (model B), whereas δ^{15}N was best modeled with c = –1

Table 2. Comparisons of ΔAIC_c among six turnover model parameterizations that treat hatchery and wild fish groups either separately or combined for each parameter and either allow for a metabolic contribution to turnover or not.

				ΔAIC_c					
Method	Isotope	Tissue	*n*	Model A	Model B	Model C	Model D	Model E	Model F
Growth-based	$\delta^{13}C$	Liver	98	**0.0**	5.4	28.6	31.4	242.6	274.6
		Blood	99	**0.0**	3.1	6.7	4.8	52.9	55.6
		Muscle	99	1.2	**0.0**	0.6	1.9	6.0	4.7
	$\delta^{15}N$	Liver	98	**0.0**	32.4	1.7	30.2	231.5	238.4
		Blood	99	**0.0**	4.3	7.7	6.0	21.6	53.8
		Muscle	99	4.6	2.7	2.9	1.1	0.7	**0.0**
Time-based	$\delta^{13}C$	Liver	101	0.7	**0.0**	9.9	8.1	270.6	273.9
		Blood	103	1.8	2.1	0.3	**0.0**	94.1	93.3
		Muscle	103	**0.0**	5.7	2.4	5.5	11.7	9.9
	$\delta^{15}N$	Liver	101	1.5	27.7	**0.0**	40.0	270.9	317.1
		Blood	103	**0.0**	12.6	1.0	13.6	54.8	60.9
		Muscle	102	2.6	1.4	0.5	1.1	2.1	**0.0**

Note: Models were fitted using the growth- and time-based methods for each isotope, tissue, and group. Brief model descriptions (see text for full details): A, group-specific turnover parameter (*c* or *m*), group-specific δ_f; B, group-specific turnover, single δ_f; C, single *c* or *m*, group-specific δ_f; D, single *c* or *m*, single δ_f; E, no metabolic contribution to turnover ($c = -1$ or $m = 0$), group-specific δ_f; and F, no metabolic contribution to turnover, single δ_f. The best model for each comparison is indicated in bold type.

(model F). The best parameterizations for time-based modeling were inconsistent across isotope–tissue combinations, with models A, B, C, D, and F selected as the best model at least once (Table 2). All subsequent parameter estimates and model fits presented were based on the best parameterizations identified in Table 2 using ΔAIC_c.

Turnover and fractionation of $\delta^{13}C$

Isotopic turnover of $\delta^{13}C$ in summer flounder tissues was clearly evident over the duration of the experiment and well represented by both growth- and time-based models (Figs. 3, 4). Initial $\delta^{13}C$ values of hatchery and wild fish were similar and approximately 10‰ removed from the mean initial isotopic signature of the krill diet (Table 3). For a given group, turnover was most rapid in liver followed by blood then muscle, as indicated by the more negative values of *c* (growth-based model; Table 4) and higher values of *m* (time-based model; Table 5). However, the magnitude of these parameters for liver and blood were similar, translating into small differences in isotopic trajectories by growth (Figs. 3*a*, 3*b*) or over time (Figs. 4*a*, 4*b*).

Half-lives for $\delta^{13}C$ in liver, blood, and muscle were estimated as 20, 22, and 69 days for hatchery fish and 17, 23, and 49 days for wild fish, respectively (Table 5). A 95% isotopic turnover in liver and blood could occur in as little as 72 or 94 days, whereas 95% turnover in muscle could require up to 298 days. In terms of growth, hatchery fish attained 50% turnover in liver, blood, and muscle after growing 1.3, 1.4, and 2.7 times their initial size, respectively (Table 4). Wild fish needed to grow 1.1, 1.2, and 2.5 times their initial size to attain 50% turnover in liver, blood, and muscle, respectively.

For blood and liver, estimates of the asymptotic isotopic value, δ_f, were relatively consistent between modeling methods and between experimental groups. Differences in δ_f estimates between the time-based and growth-based models were relatively small (<0.8‰) for blood and liver, and H and W groups differed by less than 1‰ (Tables 4, 5). Unlike liver and blood, there was a higher degree of variability in δ_f estimates for muscle, with larger differences up to ~4‰ between estimates. The uncertainty in these estimates was reflected in the higher SE of these parameters.

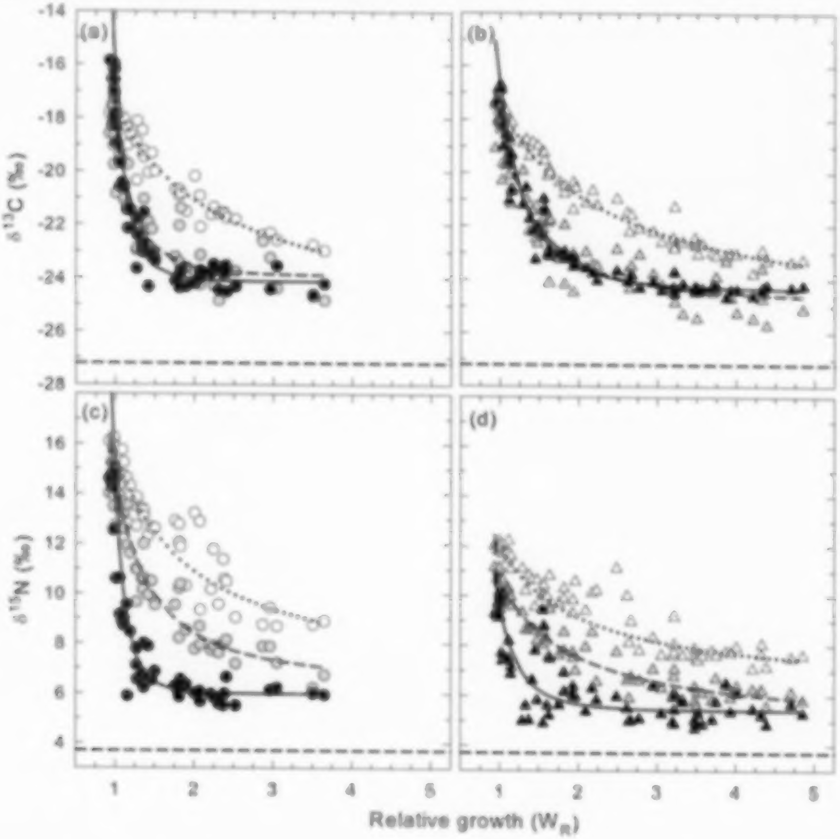
Fractionation estimates for $\delta^{13}C$ varied by tissue, group, and the model used. Liver fractionation values were the most consistent, ranging from 2.76 to 3.05‰ (Tables 4, 5). Fractionation estimates for blood ranged from 2.34‰ to 3.27‰, whereas muscle fractionation varied from 0.71‰ to 4.79‰.

Turnover and fractionation of $\delta^{15}N$

Isotopic turnover of $\delta^{15}N$ in summer flounder tissues was also well represented by growth- and time-based models. Hatchery fish exhibited more depleted initial $\delta^{15}N$ values than wild fish due to the different initial diets of each group; initial values were between 5.9‰ and 11.9‰ more $\delta^{15}N$-enriched than the krill diet (Table 3). Turnover rates of $\delta^{15}N$ reflected the same general patterns seen for $\delta^{13}C$, with liver rates being the fastest (Figs. 3*c*, 3*d*, 4*c*, 4*d*). Values for *m* were more than four times greater for the liver than for the other tissues (Table 5). Half-life was about 10 days for liver compared with approximately 38 and 96 days for blood and muscle, respectively. Growth-based estimates of *c* also indicated a much more rapid turnover in liver, such that a 95% turnover to the new krill diet could occur after an approximate doubling in weight (Table 4). Turnover rates of $\delta^{15}N$ for blood were intermediate between liver and muscle, with muscle consistently responding the slowest to the dietary change. Muscle required over 367 days (Table 5) or over a 20-fold increase in weight (Table 4) to attain 95% of $\delta^{15}N$ turnover, given the observed growth rates.

Estimates of liver δ_f and muscle δ_f were similar across methods, whereas estimates of blood δ_f were less consistent (Tables 4, 5). For example, blood δ_f differences between W and H groups were approximately 1.6‰. Again, the magnitude of the difference between group or method estimates of δ_f seemed to be related to the SE of the parameter estimates.

Fig. 3. Changes in $\delta^{13}C$ and $\delta^{15}N$ of summer flounder (*Paralichthys dentatus*) tissues as a function of relative growth (W_R) after a diet switch. (*a*, *c*) Circles denote data for wild fish; (*b*, *d*) triangles represent hatchery fish. Data and growth-based model fits are shown for muscle (open symbols, dotted line), blood (shaded symbols, broken line), and liver (solid symbols, continuous line). The horizontal broken line indicates the mean value for the krill diet.



Depending on the method used and the experimental group, fractionation estimates varied from 1.45‰ to 2.28‰ (liver), 1.11‰ to 3.86‰ (blood), and 2.13‰ to 2.53‰ (muscle) (Tables 4, 5). Similar to carbon, fractionations of $\delta^{15}N$ also exhibited a group-related trend, with higher estimates for the wild group due to consistently higher δ_f values of wild fish relative to the hatchery group.

Metabolic contributions to turnover

Metabolism played an important role in isotopic turnover in liver and blood. For liver and blood, models that were parameterized to include metabolism (models A–D) were more supported by the data, yielding the lowest ΔAIC_c values (Table 2). The very large ΔAIC_c of the competing models with no metabolism (models E and F) suggest that there is practically no evidence that growth is solely responsible for isotopic turnover in blood and liver. The importance of metabolic processes to turnover in these tissues was further indicated by the significant difference of most *m* and *c* estimates from 0 or −1, respectively (Tables 4, 5). According to the growth-based model, metabolism accounted for over 46% of $\delta^{13}C$ turnover in blood, whereas metabolic contributions reached 80% for liver $\delta^{13}C$ turnover (Table 4). Metabolic contributions to $\delta^{15}N$ turnover remained comparable, except for blood of hatchery fish, which appeared to be predominantly growth-driven. Time-based models generated slightly greater estimates of metabolic contributions to isotopic turnover in blood and liver, with values generally over 69% (Table 5).

In contrast to liver and blood, isotopic turnover of muscle was primarily driven by the growth-related process of dilution. Turnover of muscle $\delta^{15}N$ was best parameterized without a metabolic contribution (model F; Table 2), with growth processes alone able to account for isotopic turnover ($D_g = 1$ (Table 4); $P_g = 1$ (Table 5)). Results for muscle $\delta^{13}C$ were less conclusive, although there was some support for growth-based parameterizations that exclude a metabolic component ($\Delta AIC_c < 6.0$ for models E and F; Table 2). Estimates of *c* for muscle $\delta^{13}C$ turnover were greater than −1 (Table 4), suggesting that turnover occurred more slowly than expected from growth, opposite to the effect that muscle metabolism would have. Time-based estimates of *m* were not significantly different from 0 for hatchery fish, and metabolism accounted for only 19% of turnover (Table 5), supporting the predominant role of growth in turnover of muscle $\delta^{13}C$.

Table 3. Mean initial isotopic values and standard deviations (SD) for tissues of hatchery (H) and wild (W) summer flounder (*Paralichthys dentatus*) and for the experimental krill diet.

		$\delta^{13}C$		$\delta^{15}N$	
Group	Tissue	Mean	SD	Mean	SD
H	Liver	−16.84	0.07	9.59	0.36
	Blood	−18.08	0.16	10.60	0.42
	Muscle	−17.48	0.12	12.01	0.15
W	Liver	−16.35	0.42	14.51	0.21
	Blood	−18.16	0.38	14.49	0.60
	Muscle	−17.48	0.37	15.55	0.57
Krill	Whole	−27.18	0.87	3.71	0.48

Discussion

Turnover and fractionation in tissues

Differences in turnover rates among the three tissues were driven by variable metabolic contributions to the turnover process. The metabolic parameters in the turnover models technically incorporate any and all turnover processes aside from growth, but there is a good physiological basis for greater metabolic turnover in blood and liver. For example, protein synthesis and degradation rates in fish livers can be one or two orders of magnitude greater than in muscle tissues that are broken down less regularly (Smith 1981; Houlihan et al. 1988; de la Higuera et al. 1999). In ginbuna crucian carp, red blood cells exhibited half-lives of ~51 days, which is slightly longer than our estimates for blood $\delta^{15}N$ turnover; however, half-lives of other blood components (white blood cells and platelets) had half-lives of <5 days (Fischer et al. 1998) and would contribute to reducing turnover in whole blood. In birds, blood plasma proteins, synthesized in the liver, have high turnover rates that translate into short half-lives of isotopes relative to muscle (Hobson and Clark 1992*a*; Hobson and Clark 1993). Some studies demonstrated little or no difference in isotopic turnover rates among fish tissues (e.g., Hesslein et al. 1993; Sweeting et al. 2005; McIntyre and Flecker 2006), but limited sample sizes may have reduced the power to detect differences in some instances. Other studies of fishes support our findings of faster liver and blood turnover relative to muscle (e.g., Suzuki et al. 2005; Logan et al. 2006; MacNeil et al. 2006; Table 6). Metabolic contributions to isotopic turnover in our study reached 79% and 91% for blood and liver, respectively, whereas turnover in muscle was predominantly mediated by growth and simple dilution as shown for most fishes (e.g., Hesslein et al. 1993; Herzka and Holt 2000). Interestingly, metabolic contributions for C turnover in blood were greater than for N turnover, reflecting different turnover rates between the isotopes. Other studies have also documented faster turnover of C relative to N in some tissues (MacAvoy et al. 2001; Suzuki et al. 2005; Guelinckx et al. 2007), likely due to different catabolic processes operating on the various biochemical constituents of tissues. Despite this, AIC and model selection techniques showed very strong support for the role of metabolism in isotopic turnover of both liver and blood, unlike muscle, and this analytical tool provides an objective means to evaluate importance of metabolism to isotopic turnover.

Trophic fractionation between prey and consumer has

Table 4. Parameter estimates (standard error (SE) in parentheses) and calculations from growth-based models of $\delta^{13}C$ and $\delta^{15}N$ turnover in summer flounder (*Paralichthys dentatus*) tissues.

Isotope	Tissue	Model	Group	c (SE)	δ_f (SE)	Δ (SE)	$G_{0.5}$	$G_{0.95}$	D_g	D_m	F_g
$\delta^{13}C$	Liver	A	H	−3.095* (0.158)	−24.42 (0.04)	2.76 (0.09)	1.3	2.6	0.40	0.60	0.99
			W	−6.532* (0.879)	−24.13 (0.09)	3.05 (0.12)	1.1	1.6	0.20	0.80	1.00
	Blood	A	H	−2.204* (0.282)	−24.84 (0.29)	2.34 (0.30)	1.4	3.9	0.54	0.46	0.97
			W	−4.123* (0.624)	−23.91 (0.26)	3.27 (0.28)	1.2	2.1	0.31	0.69	1.00
	Muscle	B	H	−0.686* (0.103)	—	—	2.7	79.0	1.27	−0.27	0.66
			W	−0.744* (0.104)	—	—	2.5	56.0	1.21	−0.21	0.62
			C	—	−26.47 (0.89)	0.71 (0.89)	—	—	—	—	—
$\delta^{15}N$	Liver	A	H	−4.023* (0.824)	5.41 (0.10)	1.70 (0.11)	1.2	2.1	0.32	0.68	1.00
			W	−7.319* (0.998)	5.99 (0.02)	2.28 (0.05)	1.1	1.5	0.18	0.82	1.00
	Blood	A	H	−1.093 (0.206)	4.82 (0.58)	1.11 (0.58)	1.9	15.5	0.94	0.06	0.82
			W	−2.085* (0.227)	6.51 (0.39)	2.80 (0.39)	1.4	4.2	0.57	0.43	0.93
	Muscle	F	H	—	—	—	—	—	—	—	0.79
			W	—	—	—	—	—	—	—	0.73
			C	−1.000	6.24 (0.04)	2.53 (0.06)	2.0	20.0	1.00	0.00	—

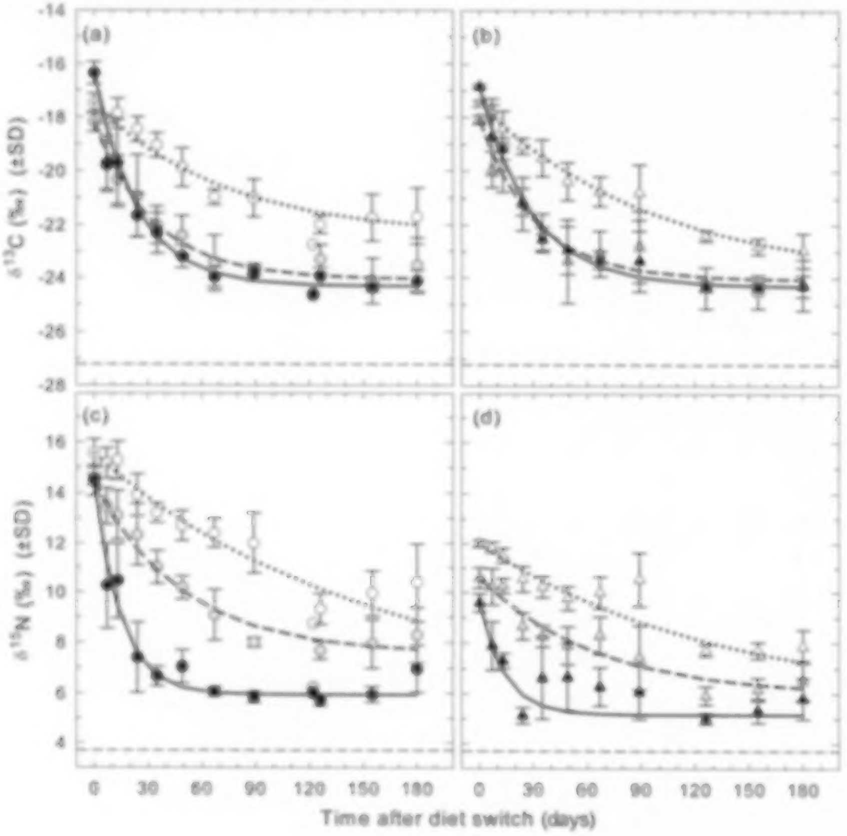
Note: See text for descriptions of models A–F. Abbreviations and parameter estimates: H, hatchery fish; W, wild fish; C, hatchery and wild fish combined; c, turnover constant; δ_f, final asymptotic isotopic value (‰); Δ, fractionation between krill diet and each tissue (‰); $G_{0.5}$, growth-based half-life; $G_{0.95}$, amount of relative growth needed to reach 95% turnover; D_g, proportion of turnover attributed to growth; D_m, proportion of turnover attributed to metabolism; F_g, fraction of complete turnover attained by the end of the experiment based on growth. Turnover constants that are significantly different from −1 ($p <$ 0.05) are marked with an asterisk (*).

Table 5. Parameter estimates (standard error (SE) in parentheses) and calculations from the time-based model of $\delta^{13}C$ and $\delta^{15}N$ turnover in summer flounder (*Paralichthys dentatus*) tissues.

Isotope	Tissue	Model	Group	m (SE)	δ_f (SE)	Δ (SE)	k'	$T_{0.5}$	$T_{0.95}$	P_g	P_m	F_t
$\delta^{13}C$	Liver	B	H	0.0261* (0.002)	—	—	0.00816	20.2	87.5	0.24	0.76	1.00
			W	0.0352* (0.003)	—	—	0.00651	16.6	71.8	0.16	0.84	1.00
			C	—	–24.32 (0.04)	2.86 (0.09)	—	—	—	—	—	—
	Blood	D	H	—	—	—	0.00816	21.6	93.5	0.25	0.75	1.00
			W	—	—	—	0.00651	22.8	98.5	0.21	0.79	1.00
			C	0.0239* (0.003)	–24.07 (0.15)	3.11 (0.17)	—	—	—	—	—	—
	Muscle	A	H	0.0019 (0.002)	–24.08 (0.50)	3.10 (0.51)	0.00816	68.9	297.9	0.81	0.19	0.84
			W	0.0075* (0.002)	–22.39 (0.35)	4.79 (0.36)	0.00651	49.4	213.4	0.46	0.54	0.92
$\delta^{15}N$	Liver	C	H	—	5.16 (0.09)	1.45 (0.10)	0.00816	9.7	42.0	0.11	0.89	1.00
			W	—	5.94 (0.08)	2.23 (0.09)	0.00651	9.9	43.0	0.09	0.91	1.00
			C	0.0632* (0.005)	—	—	—	—	—	—	—	—
	Blood	A	H	0.0076* (0.003)	5.97 (0.32)	2.26 (0.32)	0.00816	43.8	189.5	0.52	0.48	0.94
			W	0.0145* (0.003)	7.57 (0.28)	3.86 (0.29)	0.00651	33.0	142.7	0.31	0.69	0.98
	Muscle	F	H	—	—	—	0.00816	84.9	367.1	1.00	0.00	0.77
			W	—	—	—	0.00651	106.5	460.2	1.00	0.00	0.69
			C	0.0000	5.84 (0.11)	2.13 (0.12)	—	—	—	—	—	—

Note: See text for descriptions of models A–F. Abbreviations and parameter estimates: H, hatchery fish; W, wild fish; C, hatchery and wild fish combined; m, metabolic turnover constant (day^{-1}); δ_f, final asymptotic isotopic value (‰); Δ, fractionation between krill diet and each tissue (‰); k', group-specific growth rate (day^{-1}); $T_{0.5}$, time-based half-life (days); $T_{0.95}$, time needed to reach 95% turnover (days); P_g, proportion of turnover attributed to growth; P_m, proportion of turnover attributed to metabolism; F_t, fraction of complete turnover attained by the end of the experiment based on time. Metabolic turnover constants that are significantly different from 0 ($p < 0.05$) are marked with an asterisk (*).

Fig. 4. Changes in mean δ^{13}C and δ^{15}N of summer flounder (*Paralichthys dentatus*) tissues as a function of time (days) since the diet switch. (*a*, *c*) Circles denote data for wild fish; (*b*, *d*) triangles represent hatchery fish. Data (mean ± standard deviation, SD) and time-based model fits are shown for muscle (open symbols, dotted line), blood (shaded symbols, broken line), and liver (solid symbols, continuous line). The horizontal broken line indicates the mean for the krill diet.



been mostly examined for muscle, and the muscle results from this experiment were generally consistent with previous work. Our muscle fractionation estimates of Δ^{15}N (2.13‰–2.53‰) were less than the intraspecific mean value of 3.4‰ (Vander Zanden and Rasmussen 2001; Post 2002) but located well within a wide literature range (Δ^{15}N, ~ –1‰ to 6‰; McCutchan et al. 2003). Our muscle estimates of Δ^{13}C (0.71‰–4.79‰) also conformed to the broad bounds of published fractionations (Δ^{13}C, –3‰ to 4‰), but time-based estimates were more enriched than the traditionally assumed values of 0‰–1‰ (Post 2002; McCutchan et al. 2003). This discrepancy in Δ^{13}C may be largely explained by the muscle only reaching 62%–92% of equilibrium by the end of the experiment. With isotopic values not equilibrated to the diet, model estimates of δ_f were associated with relatively large standard errors that translated into reduced precision of muscle fractionation estimates. Additionally, different model parameterizations (models A–F) yielded plausible results, suggesting that the muscle Δ^{13}C fractionation estimates may not be the most robust. Future field studies planned for summer flounder in Chesapeake Bay may be able to address whether the reduced precision in muscle Δ^{13}C appears to have any influence on its accuracy.

To our knowledge, our results include the first explicit estimates of δ^{13}C and δ^{15}N fractionation for fish blood and some of the first for fish liver. In growth-based models of blood turnover, both C (2.34‰–3.27‰) and N (1.11‰–2.80‰) isotopes of flounder (hatchery and wild) were strongly fractionated from the dietary signal, more so than found in birds (Δ^{13}C, –0.3‰ to 1.2‰; Δ^{15}N, 2.2‰ to 3.1‰; Hobson and Clark 1992*b*) or seals (Δ^{13}C, 1.7‰; Δ^{15}N, 1.7‰; Hobson et al. 1996), particularly for C in wild fish. More research is needed to determine if the large blood Δ^{13}C documented for summer flounder holds for all fishes. Liver estimates for Δ^{15}N ranged from 0‰ to 3.5‰ in three studies (Trueman et al. 2005; Logan et al. 2006; Sweeting et al. 2007), corresponding with our estimates of 1.45‰–2.28‰. Due to the δ^{13}C bias introduced by the high lipid content in fish livers (Pinnegar and Polunin 1999), Δ^{13}C is best evaluated using data that were lipid-extracted or lipid-corrected, which prevents comparisons to some studies (e.g., Suzuki et al. 2005). Summer flounder in our study exhibited liver Δ^{13}C of 2.76‰–3.05‰, which was greater than that measured for rainbow trout (1.4‰; Pinnegar and Polunin 1999) or Atlantic salmon (1.6‰; Trueman et al. 2005).

Table 6. Published growth-based turnover constants (c; ± standard error (SE)) for $\delta^{13}C$ and $\delta^{15}N$ in fishes with summary data of the experimental subjects and environmental conditions, including developmental stage (H, hatchery fish; W, wild fish), initial length (L_t, ± standard deviation (SD)), and initial wet weight (W_t, ±SD).

Source	Species	Tissue	Temp. (°C)	Stage	L_t (mm)	W_t (g)	c (±SE)	
							$\delta^{15}N$	$\delta^{13}C$
A	Red drum (*Sciaenops ocellatus*)	Whole	28	Larvae, first feeding	2.6	<<0.001	−0.99±0.29	−0.96±0.35
		Whole	28	Late larvae – early juvenile	4–8	<0.001	−1.13±0.25	−1.96±0.89
		Whole	24	Late larvae – early juvenile	4–8	<0.001	−0.94±0.09	−1.12±0.32
B	Winter flounder (*Pseudopleuronectes americanus*)	Whole	13	Early juvenile	—	—	−1.00±0.22	−0.79±0.25
		Whole	18	Early juvenile	—	—	−1.14±0.34	−1.34±0.48
C	Summer flounder (*Paralichthys dentatus*)	Whole	13 and 22	Larvae, first feeding	—	0	−1.5±0.2	−1.4±0.2
		Whole	13 and 22	Larvae, artemia diet	~8	<0.001	−1.0±0.2	−1.2±0.3
		Whole	13 and 22	Early juvenile, fish diet	39	0.08	—	−3.7±1.5
D	Japanese flounder (*Paralichthys olivaceus*)	Fin	—	Juvenile	—	—	—	−4.8
		Liver	—	Juvenile	—	—	—	−11.4
		Muscle	—	Juvenile	—	—	—	−2.07
E	Mummichog (*Fundulus heteroclitus*)	Liver	18	Juvenile	40–51	0.84	−5.85±0.61	—
		Muscle	18	Juvenile	40–51	0.84	−2.33±0.25	—
F	Sand goby (*Pomatoschistus minutus*)	Heart	16.9	Juvenile	43±1	0.98	−1.55±0.83	−10.59±4.07
		Liver*	16.9	Juvenile	43±1	0.98	−15.97±4.59	−5.15±1.30
		Muscle	16.9	Juvenile	43±1	0.98	−1.19±0.57	−2.25±0.56
G	Summer flounder (*Paralichthys dentatus*)	Blood	20	Juvenile – early adult (H)	176±19	59±18	−1.09±0.21	−2.20±0.28
		Blood	20	Juvenile – early adult (W)	215±25	102±33	−2.09±0.23	−4.12±0.62
		Liver	20	Juvenile – early adult (H)	176±19	59±18	−4.02±0.82	−3.09±0.16
		Liver	20	Juvenile – early adult (W)	215±25	102±33	−7.32±1.00	−6.53±0.88
		Muscle	20	Juvenile – early adult (H)	176±19	59±18	−1.00†	−0.69±0.10
		Muscle	20	Juvenile – early adult (W)	215±25	102±33	−1.00†	−0.74±0.10

Note: Sources are as follows: A, Herzka and Holt 2000; B, Bosley et al. 2002; C, Witting et al. 2004; D, Watanabe et al. 2005; E, Logan et al. 2006; F, Guelinckx et al. 2007; G, this study. Asterisk (*) indicates that liver samples were not corrected for lipid content, which can influence $\delta^{13}C$ measurements. Dagger (†) denotes values that were not estimated but selected as the best parameterization using AIC_c.

The tissue-specific fractionations that we report are influenced by the different biochemical constituents of the tissues (e.g., amino acids, proteins, lipids), which differ in their own fractionations (DeNiro and Epstein 1977; Macko et al. 1987; McClelland and Montoya 2002). Also, assimilated dietary components are not necessarily allocated equally across all tissues, contributing to tissue-specific fractionations (Gannes et al. 1997). Additional research across a variety of taxa is needed to elucidate the variability in liver and blood fractionation factors of fishes, as has been done for muscle.

Overall, the experimental groups (wild and hatchery) yielded similar relative conclusions regarding tissue turnover rates, confirming that liver and blood respond more quickly to dietary changes than does muscle. In some cases, pooling groups was supported, generating more robust measures of model parameters, but often the groups were more appropriately parameterized separately. For example, both turnover rates and fractionations were typically higher for wild fish. Differences between wild and hatchery groups could be related to a suite of factors, including differences in initial sizes, growth rates, maturity schedules, physiology, environmental histories, and genetic diversity. The differences between groups and the potential influence of these factors are reminders that natural populations of summer flounder likely exhibit variability in both isotopic turnover and fractionation. It may therefore be more prudent for field studies on summer flounder to rely on parameter estimates derived from the wild group as opposed to hatchery fish, but we acknowledge the possibility that isotope dynamics may differ slightly between laboratory and field settings (Vander Zanden and Rasmussen 2001).

Time- vs. growth-based turnover models

Both time- and growth-based turnover models captured the patterns of isotopic changes experienced by the experimental populations, supporting the mechanistic foundations of the models in which somatic growth and metabolic tissue replacement drive isotopic turnover (Fry and Arnold 1982; Hesslein et al. 1993). However, the time-based model relies on a single growth parameter value intended to be representative of the entire population, and it is assumed to be constant through time (Tarboush et al. 2006). Summer flounder in our study experienced large variability in individual growth rates and irregular growth trajectories, characteristics that are common among fish species in natural settings. The variability in individual growth rates likely influenced parameter discrepancies between modeling methods and contributed to a large portion of the residual error in the time-based model. Not surprisingly, time-based model residuals plotted against individual growth rates consistently showed a slight negative relationship, indicating that faster growing fish experienced greater turnover after a given amount of time than slower growing individuals.

Relative to the growth-based model, time-based model turnover rates may not be as applicable to situations that deviate from the experimental conditions. Time-based turnover rate parameters (both k' and m in eq. 9) can differ by temperature and feeding rates because of the different growth rates elicited by these environmental conditions (Bosley et al. 2002; Witting et al. 2004; Watanabe et al. 2005). Turnover rates derived from growth-based models (c in eq. 3), on the other hand, may not drastically suffer from these problems because any variability in growth rates is inherently accounted for by the relative growth variable W_R. For example, Bosley et al. (2002) and Witting et al. (2004) showed that c did not vary greatly between temperature treatments, despite changes in fish growth rates, and Watanabe et al. (2005) made no indication that different feeding and growth rates influenced growth-based turnover rates (their fig. 5). Consequently, application of turnover parameters derived from growth-based models may be more suitable in field conditions where temperatures or growth rates differ from the laboratory setup from which results were generated. Although time-based models can provide valuable information, including relative differences in turnover among tissues, metabolic and growth parameters in these models may be particularly sensitive to any environmental factors that alter fish growth.

Predictive model of turnover times

Knowledge of tissue-specific turnover rates will enable researchers to select tissues suitable for the time scale of the inferences to be made in field studies utilizing stable isotopes. To avoid making an erroneous assumption that a species is in isotopic equilibrium with the sampled diet, it is important to predict the approximate isotopic equilibrium rates for different tissues, especially in migratory fishes. The half-lives calculated from the time-based turnover models may be inadequate for such predictions if field conditions, and consequently growth rates, differ from the laboratory experiment, as described above. Here, we propose a slight reformulation of the growth-based turnover model, eq. 3, to translate turnover rate constants into the more tangible unit of time for field applications.

Growth can be adequately described with an exponential model (eq. 10; Ricker 1979), and this can be substituted for W_R in the growth-based model, eq. 3, yielding

$$(14)\qquad \delta_{W_R} = \delta_f + (\delta_i - \delta_f)e^{kct}$$

For a given percentage of complete turnover (α), δ_{W_R} can be rewritten as

$$(15)\qquad \delta_{W_R} = \frac{\alpha}{100}(\delta_f - \delta_i) + \delta_i$$

For example, a 50% turnover between the initial and final isotope values would equate to α = 50% and δ_{W_R} would be half way between δ_i and δ_f. After substituting eq. 15 into eq. 14, the following model can be obtained:

$$(16)\qquad t_{\alpha/100} = \frac{\ln(1 - \alpha/100)}{kc}$$

where $t_{\alpha/100}$ is the time needed for α% turnover to be completed. The growth-based model fits presented in this paper provide the turnover rate constants, c, for three summer flounder tissues. Assuming that c is relatively constant across different specific growth rates (k), then the time needed for a given amount of turnover to occur (for a specified growth rate) can be modeled using eq. 16.

Plotting this model over a range of realistic growth rates for juvenile summer flounder (Rountree and Able 1992) using turnover estimates of $\delta^{15}N$ emphasizes the differences in temporal scales represented by various tissues (Fig. 5). For

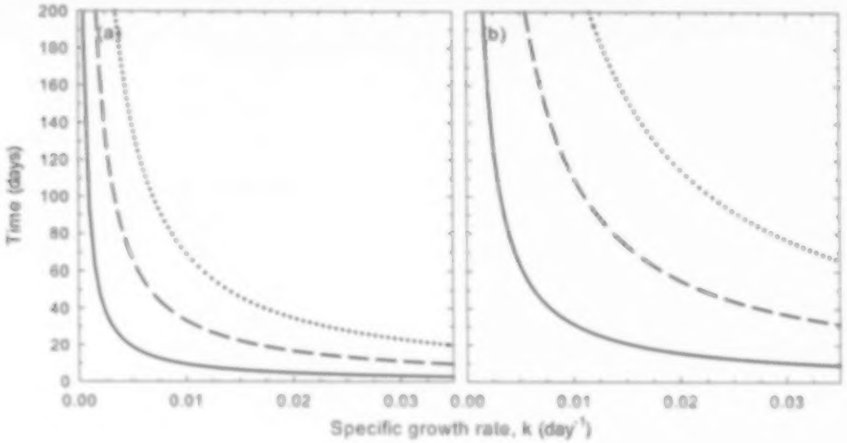


Fig. 5. Projected time to (*a*) 50% and (*b*) 90% turnover of $\delta^{15}N$ for summer flounder (*Paralichthys dentatus*) muscle (dotted line), blood (broken line), and liver (continuous line), based on eq. 16 and estimated *c* parameters for wild fish.

example, given a growth rate of 0.01 day^{-1}, muscle requires ~70 days for 50% turnover, whereas liver reaches 90% turnover in ~30 days at that growth rate. Differences in turnover times among tissues are greatest at lower growth rates, suggesting that tissue selection is particularly important for researchers working on slower growing animals. This model also highlights the important role that growth rates have on mediating the time course in which isotopic equilibrium is achieved.

The assumption that *c* is constant across different growth rates was supported by the lack of a relationship between growth-based $\delta^{15}N$ model residuals and individual growth rates, *k* (ranging from approximately 0 to 0.024 day^{-1}). Also, work on larval and juvenile flatfish did not reveal substantial differences in *c* between temperature treatments, which altered growth rates (Bosley et al. 2002; Witting et al. 2004). We did discover a slight negative relationship between growth-based model $\delta^{13}C$ residuals and individual *k*, suggesting that the consistency of *c* across growth rates may vary by isotope, but a more directed study into this would be needed.

Using eq. 16, expected turnover times can be estimated for a species of interest given information on the growth rate and turnover rate constants (known or assumed). Even though growth-based turnover rate parameters (*c*) remain unexamined for most species and tissues, ranges of possible turnover times can be estimated using published values of turnover rates for species with similar characteristics (e.g., developmental stage, size, life history characteristics) (Table 6).

Application to field studies

Large differences in tissue turnover times in this study reflect the extreme importance of tissue selection for isotopic studies of migratory fishes such as the summer flounder. Summer flounder inhabit estuarine waters such as Chesapeake Bay from spring to fall (~6–9 months) before their spawning migration to the outer continental shelf during late autumn and winter (Murdy et al. 1997). With reasonable growth rates (*k*) of 0.010–0.015 day^{-1}, muscle can require ~154–230 days to reach 90% equilibrium with a constant diet (Fig. 5). Consequently, muscle reflects dietary habits over long time periods that span the majority of typical residency of individuals in the productive estuarine waters. The lack of dietary resolution provided by muscle is compounded by the well-documented seasonal and ontogenetic dietary changes exhibited by summer flounder (Link et al. 2002; Latour et al. 2008). These dietary changes, along with regional movements, tend to exacerbate the heterogeneity of consumed isotopic signals. Such dietary variability is common for estuarine fishes in temperate environments because of the seasonality of productivity and residency in these ecosystems (Murdy et al. 1997), hindering isotopic analyses of tissues with slow turnover. Our results indicate that relative to muscle, liver and blood exhibit faster turnover rates that would benefit field studies of summer flounder and other migratory fishes by responding more rapidly to consumed material and minimizing the confounding factors introduced by movement and dietary variability. Use of blood in isotopic studies of migratory fish may be particularly useful because animals do not need to be sacrificed for sampling, allowing repeated sampling. Combining isotopic analysis of blood with other techniques such as telemetry could present a powerful means for examining habitat utilization, growth, and trophic ecology in mobile species.

In the absence of species- and situation-specific turnover rates and fractionations, certain considerations can help reduce errors when researchers use literature values in their studies. Although we advocate that field studies using stable isotopes can strongly benefit from complementary laboratory research on turnover rates and fractionations, we acknowledge that it may be impractical in some cases. Given the documented influences of species (Deniro and Epstein 1978, 1981), tissue (Logan et al. 2006), developmental stage (Sakano et al. 2005), and growth rate (Trueman et al. 2005) on turnover rates and fractionations, we recommend that researchers consider these factors, along with taxonomy, lifestyle, and habitat, when using values from previous research. However, the limited available information of this type, especially for growth-based methods (e.g., Table 6), emphasizes the importance of continued research on isotope dynamics in fishes across habitats, life stages, and tissues, especially for blood and liver.

When using literature values, it should also be common practice to conduct sensitivity analyses to reveal the potential effects that borrowed parameters may have on the conclusions drawn (e.g., Vander Zanden and Rasmussen 2001). In this regard, the parameter estimates that we present, along with eq. 16 and Table 6, may be particularly useful in estimating ranges of possible turnover times and assessing variability in dietary mixing model results. For example, given a diet shift from one prey to another, possible isotopic trajectories for a predator can be predicted using the presented turnover rates and their 95% confidence intervals. Simulations of this type suggest that within-tissue variability in dietary estimates is typically less than ~25% for flounder. Furthermore, diet estimates generated from liver and blood $\delta^{13}C$ at any given point in growth or time are indistinguishable at the 95% confidence level. For $\delta^{15}N$, on the other hand, a wild fish would require a tripling in weight or ~100 days (at a growth rate of 0.01 day^{-1}) before the 95% confidence range of diet estimates from blood would approximate (within 5%) that of the liver because of the faster turnover of nitrogen in the liver. When combined with an understanding of an organism's ecology and behavior, simulations and sensitivity analyses can aid researchers in determining the most suitable tissue and the range of appropriate inference for specific research questions utilizing stable isotopes. Specifically, deviations from the two broad assumptions of isotopic equilibrium and known fractionations can be minimized, thus reducing the sources of error in isotopic analyses, particularly in trophic studies of migratory fishes.

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Appendix A. Correcting liver $\delta^{13}C$ values for lipid content

To characterize the bias introduced into measurements of liver $\delta^{13}C$ due to lipids, 12 liver samples were divided into two aliquots and analyzed both before lipid extraction ($\delta^{13}C_{NE}$) and after lipid extraction ($\delta^{13}C_{LE}$). Lipids were extracted using a solvent mixture of 65% dichloromethane and 35% methanol (Bligh and Dyer 1959) and an accelerated solvent extractor (ASE-200; Dionex Corp., Sunnyvale, California) at 80 °C and 1800 psi. The lipid extract was concentrated to 1 mL (TurboVap 500; Zymark Corp., Hopkinton, Mass.), and its weight was determined gravimetrically to calculate the percent lipid content of each sample. Lipid-free samples were dried and analyzed for stable isotope ratios as described in Materials and methods. Following Logan et al. (2008), the relationship between elemental C:N (for nonextracted samples) and the change in $\delta^{13}C$ due to extraction ($\delta^{13}C_{LE} - \delta^{13}C_{NE}$) was evaluated using four alternative models (the numbering of equations mimics the numbering used by Logan et al. 2008):

$$\text{(A1)}\qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = D\left(\theta + \frac{3.90}{1 + 287/L}\right)$$

$$\text{where}\quad L = \frac{93}{1 + (0.246 \times \mathrm{C{:}N} - 0.775)^{-1}}$$

$$\text{(A1a)}\qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = \frac{a \times \mathrm{C{:}N} + b}{\mathrm{C{:}N} + c}$$

$$\text{(A2)}\qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = P - \frac{P \times F}{\mathrm{C{:}N}}$$

$$\text{(A3)}\qquad \delta^{13}C_{LE} - \delta^{13}C_{NE} = \beta_0 + \beta_1 \ln(\mathrm{C{:}N})$$

Each of these four models describes the relationship between the raw C:N and the bias introduced by not extracting lipids. Model A1 is based on the McConnaughey and McRoy (1979) model and includes an empirically derived expression for lipid content (L) and a parameter for the protein-lipid $\delta^{13}C$ discrimination (D). Model A1a is a generalized form of eq. A1, with the assumed values accounted for by parameters a, b, and c (Logan et al. 2008). Model A2, based on Fry (2002), has parameters for protein–lipid $\delta^{13}C$ discrimination (P) and $\mathrm{C{:}N}_{\text{lipid-free}}$ (F). And lastly, model A3 characterizes the bias due to lipids as a linear function of the log-transformed raw C:N, with $\mathrm{C{:}N}_{\text{lipid-free}}$ represented by $e^{(-\beta_0/\beta_1)}$ (Logan et al. 2008). All parameters (D, θ, a, b, c, P, F, β_0, and β_1) were estimated using nonlinear regression (SAS version 9.1; SAS Institute Inc., Cary, North Carolina). Akaike's information criterion corrected for small sample size (AIC_c; eq. 12) was used to identify the best model of the set (Burnham and Anderson 2002).

The percent lipid content of liver samples had a positive relationship with the C:N of the samples, supporting the notion that C:N is a suitable proxy for lipid content (Fig. A1). All four models captured the nonlinear trend in $\delta^{13}C_{LE} - \delta^{13}C_{NE}$ (Fig. A2). Although nearly indistinguishable from model A2, model A1 exhibited the lowest AIC_c and was identified as the best model of the set (Table A1). Consequently, all liver sample $\delta^{13}C$ from this study were corrected for lipid content using model A1 with the parameters estimated (±SE) as $D = 6.2883 \pm 1.3519$ and $\theta = 0.0612 \pm 0.1527$ (Table A1).

Fig. A1. Percent lipid content of liver samples plotted by raw, non-lipid-extracted C:N.

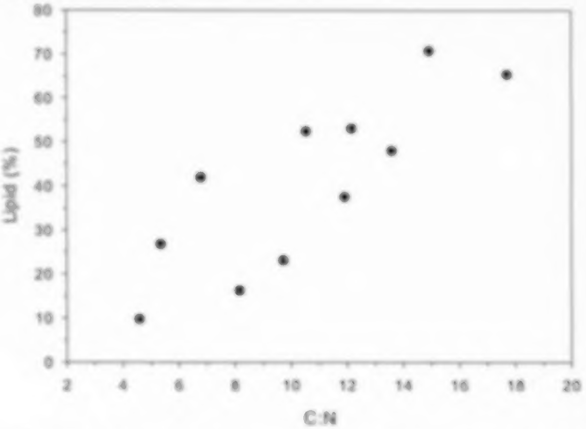

Fig. A2. Relationship between raw C:N and $\delta^{13}C$ bias introduced by not extracting lipids from liver samples. Bias in $\delta^{13}C$ was calculated as the difference between $\delta^{13}C$ for lipid-extracted samples (LE) and non-lipid-extracted samples (NE). Model fits are indicated by different lines (bold continuous line, model A1; broken line, model A1a; continuous line, model A2; dotted line, model A3). Note that models A1 and A2 are overlapping in the figure.

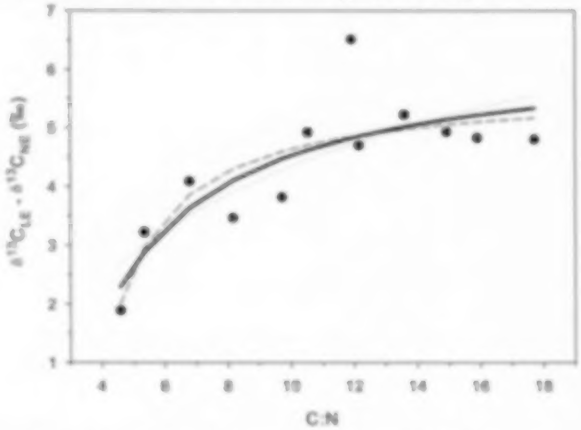

Table A1. Statistics and parameter estimates for the four competing models used to describe the relationship between liver C:N and the bias in $\delta^{13}C$ introduced by not extracting lipids.

Model	RSS	ΔAIC_c	Parameter	Estimate	SE
A1	4.778	**0.000**	D	6.2883	1.3519
			θ	0.0612	0.1527
A1a	4.537	4.093	a	5.6965	0.7851
			b	–21.7933	3.0509
			c	–2.4176	2.0722
A2	4.789	0.028	P	6.4081	0.4821
			F	2.9370	0.4410
A3	5.787	2.299	β_0	–0.5532	1.2458
			β_1	2.1277	0.5299

Note: AIC_c differences (ΔAIC_c) were calculated from residual sum of squares (RSS) for each model, and the best model is indicated in bold type. SE, standard error.

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Relative value of limnological, geographic, and human use variables as predictors of the presence of *Bythotrephes longimanus* in Canadian Shield lakes

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Abstract: The Eurasian aquatic invader *Bythotrephes longimanus* has spread rapidly throughout the Great Lakes watershed, particularly in Ontario, Canada, since its introduction in 1982. To date, the documentation of its spread has been largely anecdotal, not the result of planned surveys, so the actual distribution of the invader and the determinants of its distribution in Ontario remain uncertain. We surveyed 193 lakes in south-central Ontario to determine the importance of lake size, lake location, hydrological connection, and various physical, chemical, and human activity parameters in predicting the spread of *Bythotrephes* within the 1600-lake Muskoka watershed, North America's most heavily invaded lake region. Invaded lakes were larger than uninvaded lakes and had less acidic, more nutrient-poor, clearer waters. Contingency analyses indicated that invaded lakes were more accessible to humans and had more heavily developed shorelines. Shoreline coverage by cottages was the strongest predictor of *Bythotrephes* presence when all physical, chemical, and human use variables were included in a logistic regression model. Our results support the growing consensus that humans are primarily responsible for the spread of *Bythotrephes*.

Résumé : L'envahisseur eurasien *Bythotrephes longimanus* s'est répandu rapidement à travers le bassin versant des Grands Lacs, particulièrement en Ontario, Canada, depuis son introduction en 1982. À ce jour, la récolte des données sur cet envahissement a été en grande partie anecdotique et non le résultat d'inventaires planifiés si bien que la répartition réelle de l'envahisseur et les facteurs déterminants de sa répartition en Ontario restent incertains. Nous avons inventorié 193 lacs du centre-sud de l'Ontario afin de déterminer l'importance de la taille du lac, de sa position géographique, ses connections hydrologiques et de diverses variables physiques, chimiques et anthropiques pour la prédiction de l'envahissement de *Bythotrephes* dans le bassin versant des 1600 lacs de Muskoka, la région lacustre la plus affectée en Amérique du Nord. Les lacs envahis sont plus grands que les lacs non envahis et possèdent des eaux moins acides, moins riches en nutriments et plus claires. Des analyses de contingence indiquent que les lacs envahis sont plus accessibles aux humains et ont des rives plus fortement développées. La couverture des rives par les chalets est la variable prédictive de la présence de *Bythotrephes* lorsque toutes les variables physiques, chimiques et anthropiques sont incluses dans un modèle de régression logistique. Nos résultats appuient l'opinion de plus en plus générale qui veut que les humains sont les principaux responsables de la dispersion de *Bythotrephes*.

[Traduit par la Rédaction]

Introduction

The prevalence of nonindigenous species (NIS) in North American waters has been an issue of increasing concern over the past several decades. One such NIS is the cladoceran *Bythotrephes longimanus* Leydig (Crustacea, Onychopoda), a macroinvertebrate planktonic predator likely transported to the Great Lakes within ballast water from ships originating from the Baltic Sea (Therriault et al. 2002). Initially observed in Lake Ontario in 1982 (Johannsson et al. 1991), *Bythotrephes* was subsequently discovered in all of the Laurentian Great Lakes by 1988 (Bur et al. 1986; Lehman 1987; Cullis and Johnson 1988). Once established, *Bythotrephes* spread inland, dispersing throughout the larger, more developed lakes of the District of Muskoka, Ontario, Canada (Yan et al. 1992). To date, the species has been found in over 130 lakes throughout Ontario (Cairns et al. 2006; Francine MacDonald, Ontario Federation of Anglers and Hunters (OFAH); N.D. Yan, unpublished data), Michigan, Minnesota, and Ohio (MacIsaac et al. 2000; Branstrator et al. 2006).

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Several *Bythotrephes* dispersal mechanisms have been suggested but not yet proven. Human vectors have been inferred to be a primary method of *Bythotrephes* dispersal throughout inland basins in both Europe and central Ontario (Grigorovich et al. 1998; Muirhead and MacIsaac 2005), and gravity models indicate that recently invaded lakes experience higher boater traffic from invaded lakes and are significantly closer to all-season roads in comparison with noninvaded lakes (MacIsaac et al. 2004; Muirhead and MacIsaac 2005). Natural dispersal methods may also contribute to the spread of *Bythotrephes* (Therriault et al. 2002). Advective transport by rivers or streams could play an important dispersive role within a watershed, as resuspended resting eggs or live individuals could be transported downstream (Grigorovich et al. 1998). There is also the possibility of active upstream or downstream dispersal via the egestion of resting eggs from the digestive tracts of fish (Jarnagin et al. 2000), on the hulls of boats (S. Arnott, Queen's University, Department of Biology, Kingston, Ontario, unpublished data), or in the feathers or digestive tracts of birds (Figuerola and Green 2002).

We do not have an accurate picture of the contemporary distribution of *Bythotrephes* in Ontario. Models based either on the characteristics of European lakes that support *Bythotrephes* (MacIsaac et al. 2000) or on human dispersal vectors (MacIsaac et al. 2004) have failed, on occasion, to accurately predict the presence or absence of *Bythotrephes* in Ontario lakes. MacIsaac et al.'s (2000) model indicated that lake area and Secchi depth were the best predictors of *Bythotrephes* presence in Europe. However, recent sampling in Ontario suggests that the models based on clarity have underestimated the range of *Bythotrephes* in Canada (Cairns et al. 2007). The mesotrophic and eutrophic lakes used to build MacIsaac et al.'s (2000) model are quite different from the mostly oligotrophic lakes of the Canadian Shield where *Bythotrephes* is currently spreading. They are usually smaller, and Secchi depth is determined by water colour, not by nutrient concentrations; hence, there is no inherent reason why this model should be an accurate predictor of the presence of *Bythotrephes* in Canadian Shield lakes (Branstrator et al. 2006). MacIsaac et al.'s (2004) human dispersal model was based on boater questionnaires and need not return data that would fit an ideal probability-based design. To improve current models of *Bythotrephes* spread in Canada, more information about its current distribution and the potential for its active or passive hydrological dispersal is clearly needed. Although humans (MacIsaac et al. 2004), waterfowl (Charalambidou et al. 2003), and fish (Jarnagin et al. 2000) have all been investigated as potential dispersal vectors, to date there has been no formal investigation in Canada (i.e., one based on a survey of randomly selected lakes or of lakes selected in linked chains) into the potential for *Bythotrephes* to disperse throughout a connected hydrological landscape and among lakes that vary in size.

Here we quantify the current distribution of *Bythotrephes* in the region of Ontario with the oldest documentations of invasion, and we test the effectiveness of several variables that are included in current models predicting *Bythotrephes* spread. With new data from a survey of almost 200 lakes, we first tested the MacIsaac et al. (2000) prediction based on European data that *Bythotrephes* is more commonly found in large lakes. We also tested the potential role of active or passive spread in an interconnected landscape by sampling lakes in hydrological chains with increasing separation from lakes known to be invaded as of 2004. We determined which among water quality, morphometric, or physical variables might affect *Bythotrephes* distribution. Lastly, we collected first-hand information on boating activity, access, and shoreline development on all lakes to determine if intensity of human use of lakes (human use variables) could predict the presence of *Bythotrephes* in the most heavily invaded lake region in North America — the Muskoka region of south-central Ontario, Canada.

Materials and methods

Study area

Sampling for *Bythotrephes* was conducted on 193 lakes within watershed 2EB (Cox 1978), which contains approximately 1636 lakes of >1 ha (Cairns et al. 2006) and encompasses the District of Muskoka and parts of the counties of Haliburton, Nipissing, and Parry Sound (Ontario, Canada). This area is mostly underlain by the granitic bedrock that typifies the Precambrian Shield. Lake morphometry varies greatly with landscape position within the watershed (Quinlan et al. 2003). The 193 study lakes were largely nutrient poor (4–17.4 $\mu g \cdot L^{-1}$ total phosphorus (TP)) and softwater (17.4–98 $\mu S \cdot cm^{-1}$ conductivity) but ranged widely in dissolved organic carbon concentrations (DOC: 2–33.4 $mg \cdot L^{-1}$) and size (6.6–155.5 ha), as is typical of Canadian Shield lakes. The lakes also had a large range of boater accessibility and traffic, as would be expected from their varying sizes and landscape positions (Reed-Andersen et al. 2000). The 2EB watershed was an ideal area in which to conduct this research because it has the longest *Bythotrephes* invasion history for any lake region in North America (Yan et al. 1992) and has been suggested to be a hotspot for new invasions due to the high amount of recreational boater traffic (MacIsaac et al. 2004). As of 2004, 23 lakes within this watershed were known to be invaded by *Bythotrephes* (Cairns et al. 2006).

Survey design and lake selection methods

In 2005, 92 lakes were selected from a database of all lakes within the watershed, compiled from Landsat digital satellite data (Hélie et al. 1993). We used spatial location and lake size as strata in an equally stratified random sampling design (Hirzel and Guisan 2002). To effect this design and to ensure that the entire watershed was sampled, the study area was divided into a 12-cell grid. Within each cell, lakes were grouped into three size categories (1–10, 10.1–100, and >100 ha) and an equal number of lakes from each size category were randomly selected. This provided a set of lakes with a preset variation in size and a wide but uncontrolled variation in chemistry (Neary et al. 1990), which allowed us to determine any differences in water quality between invaded and uninvaded lakes. In 2006, 101 lakes were selected along gradients of hydrological connection to lakes known to be invaded before 2004. Most study lakes (88%) had to be located upstream of an invaded lake, as the majority of known invaded lakes are located at the bottom of their subwatersheds. However, as active upstream dispersal via fish, waterfowl, or boats is theoretically possible, patterns of upstream presence warranted investigation. These lakes were categorized as either upstream or downstream of an invaded lake and assigned a lake "chain number" — an ordinal variable reflecting the number of linked lakes in a chain of interconnected lakes that separates it from a previously known invaded lake. Chain numbers ranged from 1 to 7. The majority (75%) of sampled lakes were within chains of either one or two lakes, but 12% of the study lakes were within chains of three lakes, and 10% were among four or more lakes in a chain connected to an invaded lake. The 23 lakes in the watershed known to be invaded prior to 2005 were excluded from the list of lakes that we sampled in 2006.

Sampling protocol

Samples were collected between June and August of 2005 and 2006 as peak *Bythotrephes* abundance generally occurs in mid- to late summer (Yan et al. 2001). Lakes were sampled using a standard OFAH *Bythotrephes* conical tow net (30 cm diameter aperture, 65 μm mesh). Three samples with this net taken at different sites on a lake, each a pooled composite of two hauls, have been shown to detect *Bythotrephes* 100% of the time at typical summer abundances (4.17 animals·m^{-3}) and at least 84% of the time at low abundances (0.92 animals·m^{-3}, Boudreau and Yan 2004). To improve detection of small populations, two hauls were taken and subsequently combined into a composite sample at each of five sites along the longest fetch of each lake. To account for potential spatial irregularity of *Bythotrephes* due to wind-induced movement (Zozulya 1978), a sixth oblique tow was taken at the downwind area of each lake. Samples were preserved in 5.5% sucrose–formalin and examined in their entirety for the presence and numbers of *Bythotrephes*.

Vertical temperature, oxygen profiles, and Secchi transparency were measured at every lake at the deepest site along the sampled fetch. Composite (epilimnetic + metalimnetic) water samples were collected at this site for subsequent chemical analysis at the Ontario Ministry of the Environment's Dorset Environmental Science Centre chemistry lab (Dorset, Ontario). Samples were analyzed for pH, alkalinity, TP, calcium, conductivity, and DOC. This parameter list was selected to allow for the possibility that *Bythotrephes* presence might be affected by its acid sensitivity, limitations by food availability, and (or) detection by visual predators.

Human use variables and observations of shoreline activities that might affect *Bythotrephes* delivery were assessed at each lake. We were interested in scoring metrics related to boat access, human activity, and shoreline development density (called "cottage proportion"). As recreational boater traffic has been predicted to be a primary method of dispersal for *Bythotrephes* (MacIsaac et al. 2004), the types of access and activity for motorized watercraft were assessed. These metrics were determined by direct observations during the visit to each lake. Cottage proportion was estimated visually as the fraction of the entire shoreline that was developed with seasonal cottages or permanent dwellings. Visual shoreline estimates were subsequently corroborated with satellite views using Google Maps (http://maps.google.ca/). Boat access was scored into three categories: no motorized boat access (no boat launch that could accommodate a motorized boat of any size), public access (any boat could use the available launch(es) without restriction or with only minimal payment), or private access only (boat launches were either locked or were not available to the public because they were on private property). Activity was also divided into three categories: motorized watercraft present (any type of motorized watercraft was observed on the lake), no motorized watercraft (no evidence of any motorized activity), or the presence of a youth camp and (or) resort (could be either motorized or nonmotorized activities). The third category including lakes with camps and (or) resorts was included due to the likely higher volume of within- and among-lake boater traffic that these lakes experience.

Great care was taken to prevent our transport of *Bythotrephes* or any other NIS between lakes. Ropes, nets, and lines were never re-used during the same day. Used equipment was disinfected with a 10% bleach solution and hung to dry on a daily basis. Canoes and paddles were disinfected after each use, and motorized boats were never launched at two separate lakes in a single day. Motorboats were also power-sprayed and allowed to dry for a minimum of three days before being relaunched. Separate equipment was set aside for use in provincial parks at the direction of park authorities.

Data analysis

All data were analyzed using Sigmastat (ver. 3.11; Systat Software Inc., Chicago, Illinois), except principal component analysis (PCA) models, which were generated using CANOCO (ver. 4.1; ter Braak and Smilauer 1998), and logistic regression models, which were generated in SAS (ver. 9.1; SAS Institute Inc., Cary, North Carolina). A relatively small number of lakes, which were clear outliers in water chemistry, were removed to reduce the influence of very atypical lakes on subsequent analyses and to permit the analyses to reflect the main environmental gradients among lakes.

MacIsaac et al.'s (2000) hypothesis that *Bythotrephes* is found primarily in larger lakes was tested using logistic regression analysis to determine if the probability of presence of *Bythotrephes* in the 92 lakes sampled in 2005 was predictable from, and increased with, lake size. To further explore this hypothesis, the 99 lakes sampled in 2006, as well as the combined 2005–2006 data set, were subjected to the same analysis. To determine if there was a hydrological pattern of spread, the variable upstream–downstream location (landscape position in relation to a known invaded lake) was compared between invaded and uninvaded lakes sampled in 2006 using a Fisher's exact test. Lake invasion was compared among lakes with different chain numbers using a chi-square analysis.

To determine the most important environmental variables affecting the distribution of *Bythotrephes*, we began by performing a PCA on the correlation matrix of DOC, pH, calcium, TP, alkalinity, conductivity, lake area, surface temperature, Secchi depth, and maximum sampling depth (ter Braak and Smilauer 1998). Because of the generally non-normal distributions, all data were ranked for use in the ordination model. The sample scores from the first three axes of the ordination model were then used in a stepwise logistic regression model to determine the combination of components that best predicted *Bythotrephes* presence. The means of all 10 physical and chemical variables were also compared between invaded and uninvaded lakes sampled in both 2005 and 2006 using t tests. Mann–Whitney U tests were used for variables that did not exhibit a normal distribution before or after conventional transformation. False detection rate corrections (Benjamini and Hochberg 1995) were applied to significant physical and chemical variables to account for the possibility of type I errors.

We compared the human use variables between invaded and uninvaded lakes using a chi-square analysis. If significant, activity and boat access scores were then used, along with cottage proportion, in a logistic regression model as predictors of *Bythotrephes* presence.

To determine the combination of factors that best explained the presence of *Bythotrephes* in our study lakes, a stepwise logistic regression model was built using all significant physicochemical (combined as axes 1 and 2) and human use variables.

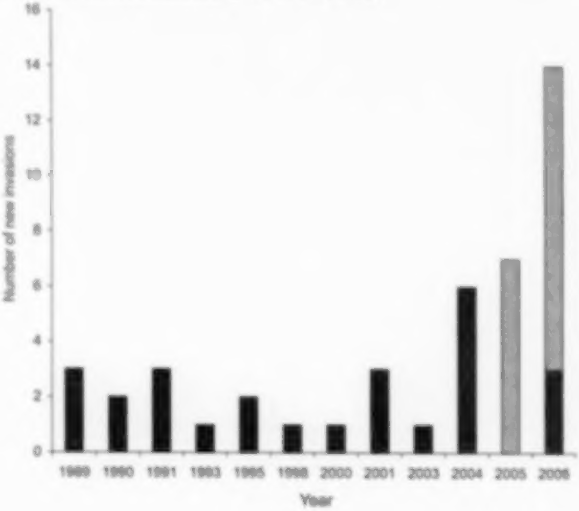
Results

With 18 invasions newly discovered in 2005 and 2006, it is clear that increased sampling effort has resulted in increased detection of *Bythotrephes* invasions in watershed 2EB (Fig. 1). The number of invaded lakes in the Muskoka region totaled 46 in 2006 (Cairns et al. 2006), approximately 3% of the lakes in the watershed. In general, invaded lakes discovered in 2005 and 2006 were spread across the study area, with the exception of provincial parks and other protected areas that appeared to be free of *Bythotrephes* in 2006 (Fig. 2).

Although MacIsaac et al. (2000) predicted that *Bythotrephes* would "prefer" larger lakes, our ongoing discovery of *Bythotrephes* in several smaller lakes in the watershed (e.g., Henshaw Lake at 25.46 ha, Silver Lake at 52.38 ha, and Silver Lake (Gravenhurst) at 53.75 ha) has resulted in a considerable size range of invaded lakes (25.46–834.30 ha). However, logistic regression models indicated that lake size was a very good predictor of *Bythotrephes* presence using the 2005 data that were sampled for this purpose (p = 0.0098) and also the 2006 (p = 0.0024) and 2005–2006 combined data sets (p = 0.001) (Fig. 3).

There did not appear to be a trend between hydrological connections and invasion. Contingency analysis of chain number (p = 0.153) and upstream–downstream location (p = 0.655) did not detect a difference between invaded and uninvaded lakes. Although only 12% of lakes were located downstream of invaded lakes, invaded lakes were relatively equally proportioned upstream and downstream from a lake known to be invaded in 2004, and there was a (nonsignificant) trend for invaded lakes to be one or two lakes away from a previously identified invaded lake. No invaded lakes were located more than two lakes away from a known invaded lake. Hence, there are clusters of invaded lakes throughout the region.

Fig. 1. Time course of the number of new *Bythotrephes* invasions discovered in watershed 2EB (Muskoka, Ontario, Canada; Cox 1978) within this study (shaded bars) and all previous studies (solid bars) since 1989. Data sources are Yan et al. (1992), Cairns et al. (2006), and personal communications from N.D. Yan, H.J. MacIsaac (Department of Biological Sciences, University of Windsor, Windsor, ON N9B 3P4), M.E. Palmer (Department of Biology, York University, Toronto, ON M3J 1P3), and F. MacDonald (Ontario Federation of Anglers and Hunters, 4601 Guthrie Drive, P.O. Box 2800, Peterborough, ON K9J 8L5).

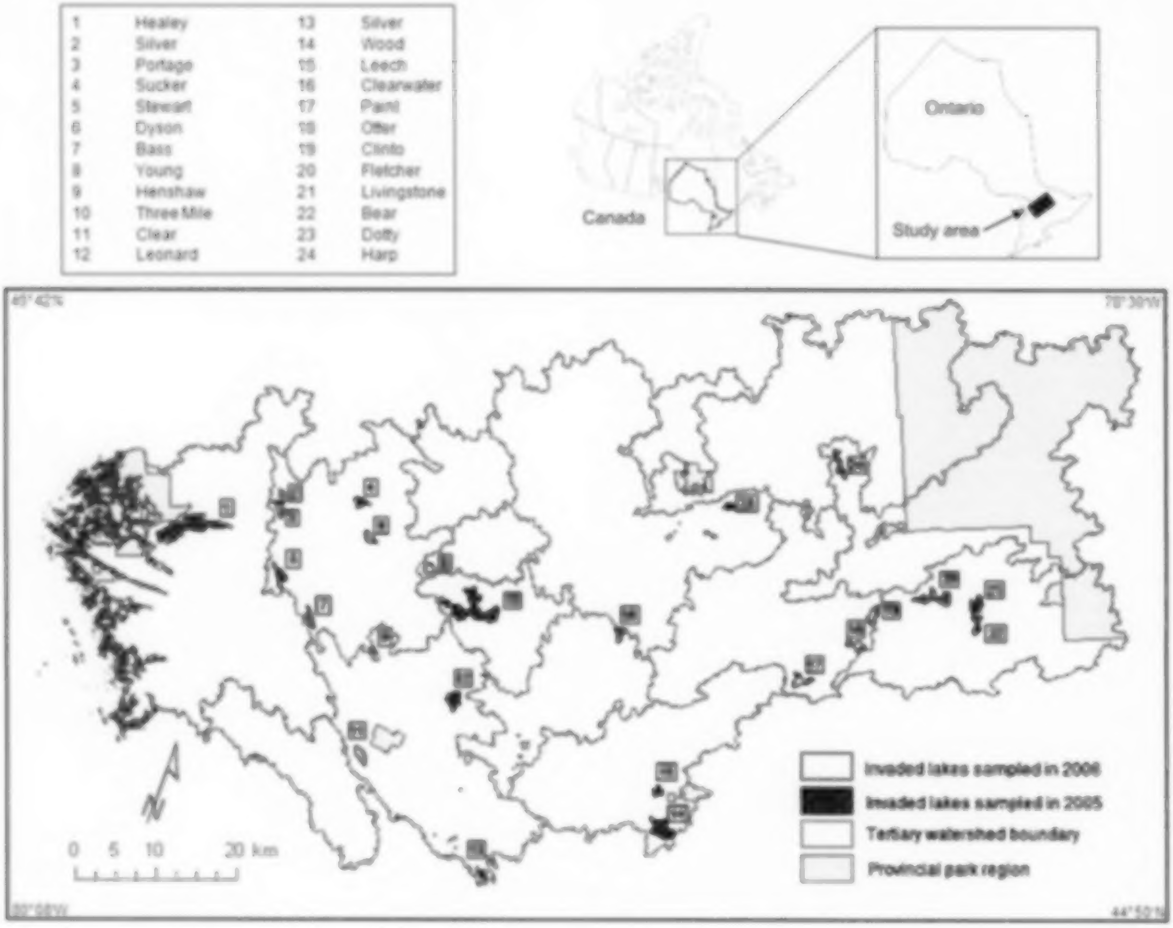


Multivariate analysis of 10 physicochemical variables revealed several strong trends. The first three components of the PCA explained 71% of the variance of the chemical and physical characteristics (Table 1). Axis 1 explained 31.5% of the variance and was most strongly represented by alkalinity, pH, calcium, and conductivity. Axis 2 explained 27.8% of the variance and was most strongly represented by TP, DOC, and maximum sampling depth. Although surface temperature strongly influenced axis 3 (score of 0.7157), this axis explained only 11.3% of the variance and surface temperature did not differ between invaded and uninvaded lakes (t test, p = 0.301). Therefore, axis 3 scores were not considered further as potential predictors of the distribution of *Bythotrephes*.

A scatterplot of axis 1 versus axis 2 scores of the physicochemical PCA revealed a cluster of invaded lakes in the upper and part of the lower left quadrant, representing lakes greater in size, depth, and Secchi depth and with lower DOC and phosphorus levels (Fig. 4). This region of the plot also contained lakes with higher pH, calcium, conductivity, and alkalinity. A stepwise multiple logistic regression model using the sample scores from the first two components indicated that axis 1 best predicted *Bythotrephes* presence (p < 0.0001), followed by axis 2 (p = 0.0023). All of the physicochemical parameters represented by axes 1 and 2 correlated with *Bythotrephes* presence (Table 2). Application of the false detection rate correction to the post hoc test with these nine variables did not change any conclusions about the significance of the relationships.

Human use differed greatly between invaded and uninvaded lakes and proved to be a key predictor of *Bythotrephes* presence. A greater proportion of invaded lakes (96%) had motorized watercraft and (or) a youth camp or resort

Fig. 2. Lakes invaded by *Bythotrephes* sampled in this study in 2005 and 2006 (Muskoka, Ontario, Canada). Map generated from the Ontario Ministry of Natural Resources (OMNR) Natural Resources and Values Information System (NRVIS, 2006) database and the National Topographic Data Base (NTDB) (Edition 3, 1999). Map based on North American Datum of 1983 (NAD83) and the Universal Transverse Mercator coordinate system.


1 Healey
2 Silver
3 Portage
4 Sucker
5 Stewart
6 Dyson
7 Bass
8 Young
9 Henshaw
10 Three Mile
11 Clear
12 Leonard
13 Silver
14 Wood
15 Leech
16 Clearwater
17 Paint
18 Otter
19 Clinto
20 Fletcher
21 Livingstone
22 Bear
23 Dotty
24 Harp
Canada
Ontario
Study area
45°42'N
78°30'W
80°08'W
44°50'N
0 5 10 20 km
Invaded lakes sampled in 2006
Invaded lakes sampled in 2005
Tertiary watershed boundary
Provincial park region

than uninvaded lakes (43%, $p < 0.0001$), and invaded lakes almost always had either a public or a private boat launch (96%, $p < 0.0001$). Cottage proportion ($p < 0.001$), activity type ($p = 0.0067$), and boat launch type ($p = 0.0078$) were all good predictors of *Bythotrephes* presence.

Clearly, many variables had the potential to differentiate invaded from uninvaded lakes and to predict the pattern of *Bythotrephes* invasion in the Muskoka region. A stepwise multiple logistic regression model, including the sample scores for the first two components of the PCA, the variables of cottage proportion, boat access, and activity type as potential independent variables, found that it was the combination of cottage proportion ($p < 0.0001$), axis 1 ($p = 0.0006$), and axis 2 ($p = 0.0073$) scores that best explained the presence of *Bythotrephes* in this watershed. In 2005 and 2006, *Bythotrephes* was found much more often in heavily developed, large, deep, clear water lakes with higher pH, alkalinity, conductivity, and calcium.

Discussion

Range expansion and dispersal

Previous models predicting *Bythotrephes* spread had potential bias as they were not built using data from probability-based designs and were constructed using lakes with very different water quality than those typifying the Canadian Shield. However, the large number of new discoveries of *Bythotrephes* that we report in the Muskoka region supports MacIsaac et al.'s (2000) prediction that many Canadian Shield lakes provide a good environment for this exotic predatory cladoceran. Our results provide strong support given that they are based on a probability-based design and because they represent the first account of the distribution of *Bythotrephes* using a sampling protocol that is proven to detect the invader at low abundances (Boudreau and Yan 2004). *Bythotrephes* is continuing to spread, and although once restricted to only larger lakes in Muskoka, it

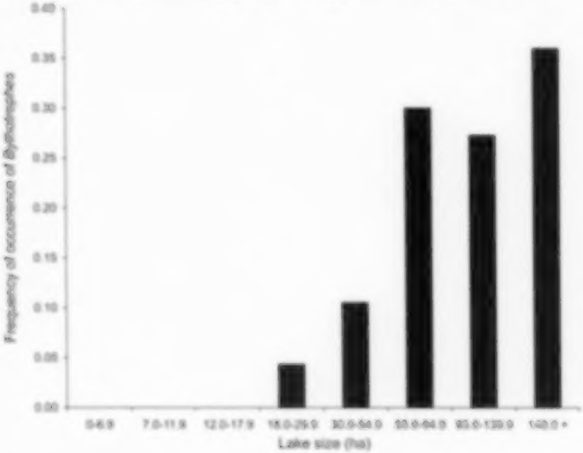


Fig. 3. Histogram of *Bythotrephes* frequency of occurrence vs. lake size categories in the 166 selected study lakes. Note the irregular ordinal *x*-axis bins, a product of the calculation of the histogram for lake size bins representing groups of approximately 20 lakes each.

is now present in lakes as small as 23 ha (Virtue Lake). Similar surveys done to track the distribution and spread of the exotic cladoceran *Daphnia lumholtzi* have also found that lake size was a very important determinant of the distribution of the species (Dzialowski et al. 2000; Havel et al. 2002). However, local factors have also been predicted to influence the presence of both *D. lumholtzi* (Havel et al. 2002) and *Bythotrephes* (MacIsaac et al. 2000; Yan et al. 2001; Branstrator et al. 2006).

The invaded lakes discovered in 2005 and 2006 were widespread in the watershed, indicating either that there are few barriers to dispersal or that the means of dispersal are very effective. Because only a small portion of our sampled lakes were located downstream of known invaded lakes, we cannot comment on the risk of passive, downstream movement of *Bythotrephes*. However, dispersal in lake chains cannot be entirely discounted, as invaded lakes were clustered in short, two or three group lake chains. Active dispersal among lakes via fish, waterfowl, or boaters could be one mechanism to explain these patterns. *Bythotrephes* is obviously capable of downstream dispersal via large rivers. Older, indirect evidence for this is found in the invaded system of Peninsula, Fairy, Mary, and Muskoka lakes (Yan et al. 1992) within the watershed. However, direct evidence also exists. *Bythotrephes* has been collected in both the Otanbee River near Peterborough (Ontario, Canada) and in the Rainy River (northwestern Ontario, Canada) (Linda Wall, Ontario Ministry of Natural Resources, P.O. Bag 3020, South Porcupine, Ontario, Canada, unpublished data). However, the very strong correlation between *Bythotrephes* presence and accessibility, activity, and cottage density clearly implicates human-mediated mechanisms of dispersal in this invader's continued spread.

If biotic methods of dispersal such as waterfowl or fish had played the primary, current role in the spread of *Bythotrephes* in this region, more invasions would have been found predominantly in undeveloped lakes, and correlation with human activity would have been much weaker. Waterfowl habitat is particularly disturbed by human activity (Dahlgren and Korschgen 1992), and thus if they had been a primary method of dispersal, a large proportion of protected, undeveloped lakes would likely have been invaded. In addition, the pattern of invasion would have been more random, as waterfowl are certainly not restricted to developed lakes (Green et al. 2002).

Overall, cottage proportion was the best predictor of *Bythotrephes* presence, which suggests that the density of waterfront homes, and consequently the density of humans, is linked with the invader's dispersal and establishment. This also suggests that local residents on the newly invaded lakes likely transported *Bythotrephes* from other invaded lakes within the region, increasing the propagule pressure to that lake. The local residents also could be transporting propagules to other noninvaded lakes, further spreading *Bythotrephes* within the region. This "stepping-stone" pattern of invasion was predicted by MacIsaac et al. (2004) and is supported by the fact that invaded lakes tend to be close together. The possibility of long-distance dispersal to more distant lakes is much lower, consistent with decreased propagule pressure. There is strong evidence that the *Bythotrephes* invasion sequence is directly related to the magnitude of the propagule pressure from invaded sources (MacIsaac et al. 2004), and propagule pressure is a good predictor of species invasiveness and community invasibility (Borbely 2001; Leung et al. 2004). The residents of Muskoka could be supporting the ongoing *Bythotrephes* invasion by their own movements.

Although it is possible that both natural and human vectors are transporting *Bythotrephes*, others have found that human vectors, and recreational boaters in particular, are primarily responsible for the transport of NIS (Johnson et al. 2001). Boaters have been identified as the primary agents of spread of the zebra mussel (*D. polymorpha*; Johnson and Carlton 1996), Eurasian watermilfoil (*Myriophyllum spicatum*; Buchan and Padilla 2000), and *D. lumholtzi* (Havel and Shurin 2004). Surveys have shown that there is a considerable amount of boater traffic throughout Ontario, and contrary to other regions where short distance travel is more prevalent (Buchan and Padilla 1999), many boaters travel long distances (>100 km) to get to their lakes of choice (Borbely 2001). Proximity to major roads is also a factor that has been positively associated with *Bythotrephes* invasion (Borbely 2001), and the invaded lakes discovered in 2005 and 2006 have similar ease of accessibility (Cairns et al. 2007). Muirhead and MacIsaac (2005) showed that boater movement was a useful predictor of the pattern of *Bythotrephes* spread in Muskoka up to 2004. This study supports these findings and also raises the possibility of movement of propagules between closely connected lakes.

Establishment

Both univariate and multivariate analyses indicate that *Bythotrephes* tends to persist in lakes with certain chemical and physical characteristics. With the exception of surface temperature, invaded and uninvaded lakes had different means for all selected physical and chemical variables. In many cases, ranges for invaded lakes were smaller but within the ranges of uninvaded lakes. This could indicate that many lakes are vulnerable to invasion, as many Ponto-

Table 1. Eigenvalues of 10 limnological variables indicating the loading on axes 1, 2, and 3 of a principal components analysis (PCA).

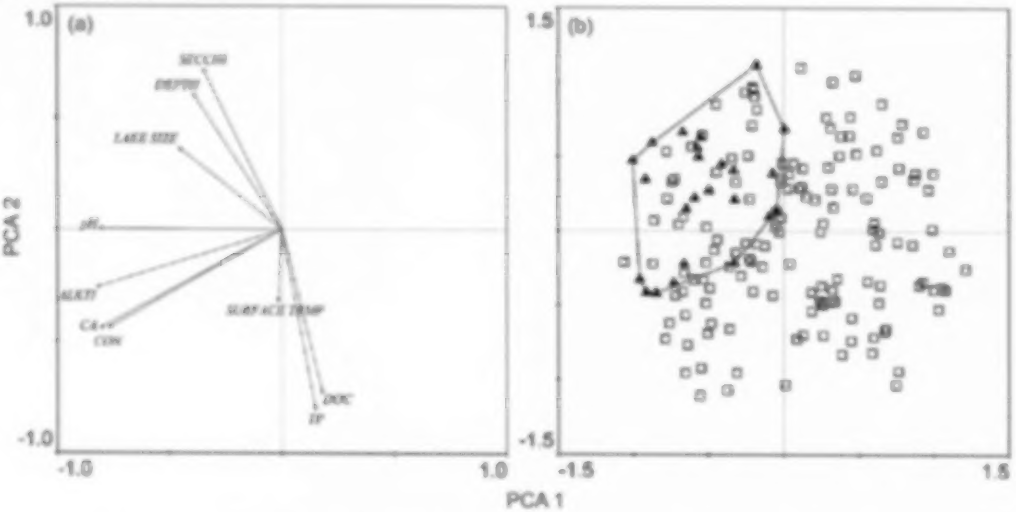
Variable	Axis 1	Axis 2	Axis 3
Lake size	–0.4597	0.3663	0.4895
Maximum sampling depth	–0.3957	**0.6083***	0.3311
Secchi depth	–0.3512	**0.7196***	–0.2800
Surface temperature	–0.0162	–0.3238	**–0.7157***
pH	**–0.8159***	0.0044	–0.0575
Dissolved organic carbon	0.1826	**–0.7362***	0.3314
Total phosphorus	0.1498	**–0.8077***	0.2512
Alkalinity	**–0.8259***	–0.2602	–0.0957
Conductivity	**–0.7745***	–0.4415	0.0488
Calcium	**–0.8098***	–0.4430	–0.0739
Variance explained (%)	31.5	27.8	11.3
Cumulative variance (%)	31.5	59.3	70.6
Logistic regression p value[†]	**<0.001**	**0.0015**	0.0553

Note: The PCA was run on the correlation matrix of these variables for 166 lakes sampled in 2005 and 2006.

*Variables were considered highly correlated with one of the three axes when scores were >0.600, indicated in bold.

[†] p values for inclusion of the axis score in a logistic regression predicting probability of presence.

Fig. 4. (*a*) Biplot and (*b*) scatterplot of sampled lakes (n = 166) based on axes 1 and 2 of a principal components analysis (PCA) carried out on 10 ranked limnological characteristics. (*a*) Variables are lake size (LAKE SIZE), maximum sampling depth (DEPTH), Secchi depth (SECCHI), pH (pH), total inflection point, or Gran, alkalinity (ALKTI), calcium (CA), conductivity (CON), surface temperature (SURFACE TEMP), total phosphorus (TP), and dissolved organic carbon (DOC). (*b*) Solid triangles represent lakes invaded by *Bythotrephes* within watershed 2EB, Muskoka region, Ontario.



Caspian species such as *Bythotrephes* tend to be very tolerant of a wide range of chemical parameters (Reid and Orlova 2002). In contrast, this could also indicate that there is a limited physicochemical range in which *Bythotrephes* can successfully establish.

Despite the statistical significance, it is our belief that *Bythotrephes* presence is not being limited by current physical and chemical variables in the watershed. Rather, we believe that these correlations are spurious and attributable to the strong relationship between invasion frequency and human use related propagule pressure. Although *Bythotrephes* is most likely unable to survive in lakes with conditions drastically different from those in its native range, the expansion of its range to lakes with higher DOC (e.g., Island lake, Duluth, Minnesota; see Branstrator et al. 2006) and that are smaller in size indicate that abiotic factors may not be important regulators of the presence of this species in many inland lakes. Alkalinity, pH, conductivity, and calcium levels tend to increase with increasing lake order, which is highly correlated with lake size within the Canadian Shield (Quinlan et al. 2003). Although our results indicate that invaded lakes have higher ranges in certain chemical variables, these associations can also be linked to their greater surface area. As lake size increases and lakes are lower in

Table 2. Comparison of means of 10 limnological characteristics of invaded ($n = 24$) and uninvaded ($n = 142$) lakes.

Variable	Mean of uninvaded lakes (range)	Mean of invaded lakes (range)	Test type	*p* value
Lake size (ha)	24.1 (1.0–623.8)	112.6 (25.5–834.3)	Mann–Whitney rank sum	**<0.001**
Maximum sampling depth (m)	8.1 (0.5–40.5)	15.0 (6.9–29.0)	Mann–Whitney rank sum	**<0.001**
Secchi depth (m)	3.8 (0.4–9.1)	4.4 (2.6–7.8)	Mann–Whitney rank sum	**0.018**
Surface temperature (°C)	24.0 (18.2–28.5)	24.3 (16.8–27.3)	*t* test	0.301
pH	6.3 (5.0–8.0)	6.7 (6.0–7.4)	Mann–Whitney rank sum	**<0.001**
Dissolved organic carbon ($mg·L^{-1}$)	5.0 (2.0–33.4)	4.3 (2.3–7.7)	Mann–Whitney rank sum	**0.015**
Total phosphorus ($\mu g·L^{-1}$)	8.5 (1.2–59.0)	5.8 (3.3–12.8)	Mann–Whitney rank sum	**0.006**
Alkalinity ($mg·L^{-1}$ as $CaCO_3$)	3.1 (-0.2–16.4)	4.7 (0.3–17.8)	Mann–Whitney rank sum	**0.004**
Conductivity ($\mu S·cm^{-1}$)	24.8 (12.4–150.0)	32.3 (17.4–132.0)	Mann–Whitney rank sum	**0.003**
Calcium ($mg·L^{-1}$)	2.3 (1.0–7.9)	2.6 (1.7–12.3)	Mann–Whitney rank sum	**0.003**

Note: Range is indicated in parentheses after the mean. Significant values after Benjamini–Hochberg false detection rate post-hoc correction are indicated in bold.

the landscape, boat access and activity also increase (Reed-Andersen et al. 2000). However, it is important to note that not all of the accessible, populated lakes invaded by *Bythotrephes* are large — Henshaw Lake is only 25 ha and Virtue Lake is 23 ha. Therefore, the strong correlation between lake size and *Bythotrephes* presence is more likely related to the increased propagule pressure that these lakes encounter, as opposed to abiotic and biotic constraints on *Bythotrephes* establishment.

Bythotrephes is not present in all populated and accessible lakes in the Muskoka region, perhaps owing to its relatively short invasion history or to unequal propagule pressure across the watershed. Alternatively, it could indicate that there is a secondary barrier to establishment that is restricting *Bythotrephes* colonization. One possible barrier could be high DOC content, as we did find that *Bythotrephes* was less likely to be present in lakes with higher DOC levels, which would lead to shallow Secchi depths and low-light visibility restrictions (Wissel 2001). However, high DOC levels have not prevented the colonization of Island Lake, Minnesota, where *Bythotrephes* thrives (Branstrator et al. 2006). Low food levels could also act as a barrier, as high springtime zooplankton biomass has been attributed to the success of *Bythotrephes* in Harp Lake (Young 2007). In addition, declining calcium levels and decreased phosphorus across the Canadian Shield (Jeziorski et al. 2008; Yan et al. 2008), when coupled with temperature increases (N. Kim, Department of Biology, York University, Toronto, Ontario, unpublished data), could have detrimental effects on the ability of *Bythotrephes* to colonize successfully. However, our knowledge of factors affecting the biology of *Bythotrephes*, including its food and calcium requirements, is insufficient to generate any firm conclusions.

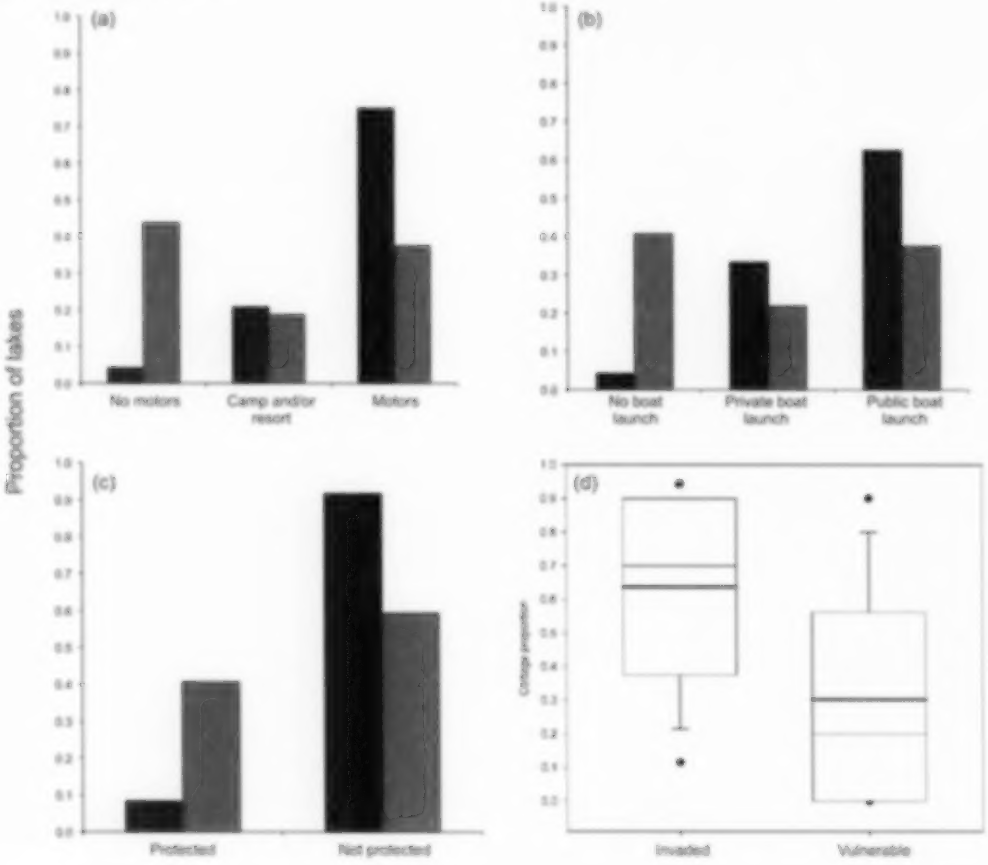
The invasion of inland lakes in Ontario is still at an early stage. Although it is theoretically possible that one individual could spawn a new invasion (Drake et al. 2006), Allee effects might increase propagule sizes required for establishment (Leung et al. 2004). Generally, only a small percentage of organisms survive the initial dispersal into a new environment and most perish in transport (Lodge 1993); however, such establishment success rates are notoriously difficult, perhaps impossible, to quantify. Nonetheless, when individuals arrive in a new ecosystem, they may encounter many physical, chemical, or ecological barriers to colonization success. Seasonality also plays an important role in the success of *Bythotrephes*, as there are clear temporal "windows of invasion" opportunities that facilitate its establishment (Drake et al. 2006). Tourists and vacationers arrive in the greatest numbers in the Muskoka region from May until September, increasing the population of the area and substantially increasing the recreational boater activity. This time period also happens to be the window in which *Bythotrephes* sustains its greatest population growth. Thus, we predict that *Bythotrephes* will continue to spread in the Muskoka region and beyond.

Future management and study implications

There is no argument that the homogenization of the Earth's biota through the introduction of exotic species is having a detrimental impact on invaded ecosystems (Vitousek et al. 1997). *Bythotrephes* is no exception to this phenomenon, with biodiversity losses among zooplankton commonly recorded in invaded North American lakes (Yan et al. 2002; Barbiero and Tuchman 2004; Strecker et al. 2006). Our study supports the theory that human-mediated propagule pressure is driving the invasion of *Bythotrephes* in the Muskoka region and suggests that abiotic factors may play a less important role in its establishment success than previously predicted. Although likely still limited by chemical and physical constraints related to water clarity and food availability, *Bythotrephes* is expanding its range into lakes that have been predicted not to sustain populations. These findings present an ominous forecast for the future of Canadian Shield lakes.

One novel result of this study is that management efforts and land use designations may be responsible for the many uninvaded lakes that have ideal conditions to support a *Bythotrephes* invasion. Previous models of spread (MacIsaac et al. 2004; Muirhead and MacIsaac 2005) have not included such extensive field quantifications of restrictions to propagule introduction. Looking at this subset of "vulnerable" lakes compared with all other lakes (i.e., the uninvaded lakes within the confines of the invaded lake polygon in Fig. 4), logistic regression or contingency analysis suggests

Fig. 5. (*a–c*) Frequency histograms comparing uninvaded lakes (called "vulnerable," shaded bars) with lakes invaded by *Bythotrephes* (called "invaded," solid bars) by (*a*) activity type (lakes with no motorized watercraft, lakes with motorized watercraft, lakes with a camp and (or) resort), (*b*) boat launch type (no motorized boat launch, public boat launch, private boat launch), and (*c*) whether a lake is within a protected area (provincial park, nature reserve, private hunting area, or other). Uninvaded lakes are considered to be "vulnerable" if they are contained within the invaded lakes envelope of the principal components analysis (PCA) in Fig. 4, i.e., lakes that cannot be limnologically distinguished from invaded lakes. (*d*) Boxplot comparing invaded lakes with vulnerable lakes by cottage proportion (defined as the proportion of shoreline that is occupied by waterfront homes). The thick center line indicates the mean, the thin center line represents the median, the box represents the 25th and 75th quartiles, the error bars indicate the 10th and 90th percentiles, and the points outside the bars indicate the 5th and 95th percentiles (outliers).



that boat launch availability ($p = 0.008$), activity type ($p = 0.011$), and cottage proportion ($p = 0.003$) were all significant factors in determining why a vulnerable lake might not be invaded (Figs. 5*a*, 5*b*, 5*d*). This indicates that these lakes, which are limnologically indistinguishable from invaded lakes, might not be invaded because they experience less boater activity, have little or no boat access, and have less shoreline development than invaded lakes. A fourth variable related to human activity was created from observations of the geographic location of the lake, with lakes classified as being either "protected" or "not protected." Protected lakes were within national or provincial parks, hunting reserves, conservation areas, or private nature reserves. The protected lakes were much less likely to be invaded, despite having acceptable conditions for *Bythotrephes* to survive ($p = 0.001$) (Fig. 5*c*). This observation may have direct implications for the management of *Bythotrephes*. Protecting vulnerable lakes by restricting access and waterfront development and imposing fines on boaters that demonstrate "risky" activity (Muirhead and MacIsaac 2005) could all help prevent the further spread of *Bythotrephes*.

Whether the regions surrounding the study watershed are experiencing the same invasion rates as the Muskoka region is unknown at this time. However, several lakes in the Leslie M. Frost Nature Reserve, located southeast of watershed 2EB, are invaded (M.E. Palmer, Department of Biology, York University, Toronto, Ontario, unpublished data), which indicates that even protected areas are vulnerable. *Bythotrephes* has been reported in Lake of the Woods in northwestern Ontario and is now poised to invade lakes in eastern

Manitoba. The greatest concern with this rapid spread is the profound effects that *Bythotrephes* is known to have on zooplankton communities and perhaps indirectly on phytoplankton and higher trophic levels across the Canadian Shield.

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Empirical evaluation of predator-driven diel vertical migration in Lake Superior

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Abstract: Recent studies on Lake Superior suggest that diel vertical migration (DVM) of prey (generalized *Coregonus* spp.) may be influenced by the density of predatory siscowet (*Salvelinus namaycush*). We empirically evaluated this hypothesis using data from acoustic, midwater trawl, and bottom trawl sampling at eight Lake Superior sites during three seasons in 2005 and a subset of sites in 2006. We expected the larger-bodied cisco (*Coregonus artedi*) to exhibit a shallower DVM compared with the smaller-bodied kiyi (*Coregonus kiyi*). Although DVM of kiyi and cisco were consistent with expectations of DVM as a size-dependent, predator-mediated process, we found no relationship between siscowet density and the magnitude of DVM of either coregonid. Cisco appear to have a size refuge from siscowet predation. Kiyi and siscowet co-occur in demersal habitat > 150 m during the day, where visual predation is unlikely, suggesting predator avoidance is not a factor in the daytime distribution of kiyi. Seasonal patterns of kiyi DVM were consistent with reported DVM of their primary prey *Mysis relicta*. Our results suggest that consideration of nonvisual foraging, rather than light-based foraging theory (i.e., the antipredation window), is necessary to understand the processes driving DVM in deepwater systems.

Résumé : Des études récentes sur le lac Supérieur laissent croire que la migration verticale journalière (DVM) des proies (les *Coregonus* spp. en général) est influencée par la densité du prédateur, le touladi siscowet (*Salvelinus namaycush*). Nous évaluons cette hypothèse de manière empirique à l'aide de données provenant d'échantillonnages acoustiques et de prélèvements aux chaluts pélagique et benthique dans huit sites au lac Supérieur durant trois saisons en 2005 et dans un sous-ensemble de sites en 2006. Nous nous attendions à ce que le cisco de lac (*Coregonus artedi*) de plus grande taille ait une DVM moins profonde que le cisco kiyi (*Coregonus kiyi*) plus petit. Bien que les DVM du cisco kiyi et du cisco de lac soient en accord avec les attentes relatives à la DVM comme processus relié à la taille et géré par le prédateur, il n'existe aucune relation entre la densité du siscowet et l'importance de la DVM chez les deux corégonidés. Les ciscos semblent posséder un refuge relié à la taille contre la prédation du siscowet. Le cisco kiyi et le cisco de lac se retrouvent ensemble dans l'habitat benthique > 150 m durant le jour où la prédation visuelle est peu probable, ce qui indique que l'évitement des prédateurs n'est pas un facteur explicatif de la répartition du cisco kiyi durant la journée. Les patrons saisonniers de DVM du cisco kiyi correspondent aux patrons de DVM signalés chez leur proie principale *Mysis relicta*. Nos résultats indiquent qu'il faut tenir compte de la recherche non visuelle de nourriture plutôt que de la recherche basée sur la lumière (c'est-à-dire, la fenêtre antiprédation) pour comprendre les processus explicatifs de la DVM dans les systèmes d'eau profonde.

[Traduit par la Rédaction]

Introduction

Diel vertical migration (DVM) is a common feature in aquatic systems, occurring over multiple trophic levels including primary producers (e.g., James et al. 1992), herbivores (e.g., Stich and Lampert 1981; Frost and Bollens 1992; Thomson and Allen 2000), planktivores (e.g., Janssen and Brandt 1980; Levy 1987, Scheuerell and Schindler 2003), piscivores (e.g., Kitagawa et al. 2000; Hrabik et al. 2006; Sims et al. 2006), and omnivores (e.g., Beeton 1960). A number of mechanisms have been proposed to explain DVM behavior. These mechanisms include maximizing for-

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aging opportunities (e.g., Narver 1970; Schoener 1971; Eshenroder and Burnham-Curtis 1999), optimizing bioenergetic efficiency (e.g., Brett 1971; Bevelhimer and Adams 1993), and minimizing predation risk (e.g., Eggers 1978; Levy 1987; Clark and Levy 1988). Clark and Levy (1988) synthesized these mechanisms into a behavioral model predicting that movement to shallower depths for feeding should occur at intermediate light levels (i.e., the antipredation window) minimizing the ratio of predation risk to feeding (or growth) rate. Evidence in support of the antipredation window hypothesis has grown in recent years (e.g., Kaartvedt et al. 1996; Scheuerell and Schindler 2003; Hardiman et al. 2004).

Recent research on Lake Superior suggests that predator density impacts the DVM behavior of forage fishes in offshore regions. Observations obtained from acoustics suggest that the magnitude of DVM in *Coregonus* spp. was positively related to the density of siscowet (*Salvelinus namaycush*; Hrabik et al. 2006), a deepwater morphotype of lake trout that preys largely on coregonids and deepwater sculpin (*Myoxocephalus thompsonii*; Conner et al. 1993; Harvey and Kitchell 2000; Ray et al. 2007) and also appears to undergo DVM (Hrabik et al. 2006). Jensen et al. (2006) developed a model to examine the mechanisms underlying coregonid DVM behavior and found strategies that minimized the ratio of predation risk (μ) to potential growth rate (G) best explained their vertical distribution. Their model suggested two DVM strategies that coregonids might follow: a low risk – low growth trajectory characterized by a deeper DVM and a high risk – high growth trajectory characterized by a shallower DVM. Jensen et al. (2006) suggested that the deeper trajectory would be advantageous when predator density is high and the shallower trajectory more advantageous when predator densities are low or coregonid densities are high (i.e., greater potential for intraspecific competition). Empirical observations of coregonid vertical distribution reported by Hrabik et al. (2006) were consistent with these modeling predictions, but were limited to three sampling occasions across two years and did not include sampling of demersal habitat.

Two coregonid species are common in offshore waters of Lake Superior — kiyi (*Coregonus kiyi*) and adult cisco (*Coregonus artedi*; Hrabik et al. 2006; Stockwell et al. 2006; Yule et al. 2009). Other coregonid species exist in Lake Superior, including shortjaw cisco (*Coregonus zenithicus*) and bloater (*Coregonus hoyi*), but the former is rare (Hoff and Todd 2004; Gorman and Todd 2007) and the latter is largely confined to where bathymetric depths are less than 140 m (Adams et al. 2006). Adult cisco differ from kiyi in several basic attributes (Table 1). First, cisco reach larger sizes than kiyi as adults (Bersamin 1958; Smith 1964; Stockwell et al. 2006) and thus may be less susceptible to predation. Second, cisco feed on different prey (copepods and cladocerans) than kiyi (*Mysis relicta*). Finally, cisco are not associated with the bottom during daylight (Stockwell et al. 2006) but kiyi are (Yule et al. 2007). Based on these differences, divergent DVM behavior between the two species should be expected whereby the larger-bodied cisco will likely undergo less pronounced DVM than the smaller-bodied kiyi. The Hrabik et al. (2006) and Jensen et al. (2006) studies did not explore DVM as a species-specific process.

In this study, we use complementary sampling techniques in offshore demersal (bottom trawls) and pelagic (midwater trawls and acoustics) habitats to empirically test several hypotheses about coregonid DVM in Lake Superior. First, we compare day and night bottom trawl catches to test Hrabik et al.'s (2006) acoustic observations of DVM in siscowets and coregonids. If siscowet and their coregonid prey perform DVM, we would expect day bottom trawl catches to be higher than night bottom trawl catches (e.g., TeWinkel and Fleischer 1999). Second, Hrabik et al.'s (2006) interannual comparisons suggest that the magnitude of DVM of coregonids may be higher in years when siscowet density is high. We therefore expected a positive correlation across sample sites between the magnitude of coregonid DVM (i.e., the difference between daytime and nighttime median depths) and siscowet density. Third, we expect the larger-bodied cisco to undergo less pronounced DVM than the smaller-bodied kiyi because of diet preferences, observed daytime habitat usage differences, and likely differences in vulnerability to siscowet predation (Table 1). Consequently, we expect a strong relationship between the magnitude of kiyi DVM and siscowet density compared with adult cisco. Such a finding would suggest that species differences influence DVM behavior and may account for the two most effective strategies modeled for a generalized coregonid by Jensen et al. (2006).

Materials and methods

General sampling design

We sampled a total of eight stations distributed across Lake Superior (Fig. 1) in spring (May and June), summer (July and August), and fall (October) of 2005. Two of the sites were sampled again in 2006 during spring, summer, and fall (Table 2). Stations included in this study had bathymetric depths ranging from 147 m to 327 m (Table 2). Stations were sampled with bottom (day and night) and midwater (night) trawls and acoustic gear (day and night) using the US Geological Survey's R/V *Kiyi*. Acoustic data were collected concurrently with each trawl tow. Day samples were collected after sunrise and before sunset. Night samples were collected between 0.5 h after nautical twilight in the evening and 0.5 h before nautical twilight in the morning. In 2005, day and night sampling at each station were performed within the same 24-h period (median difference, 6 h; range, 5–19 h). In 2006, time between day and night sampling at each station was greater because of logistical constraints (median difference, 54 h; range, 4–336 h). Only two sites were sampled in 2006 because of budget shortcomings (Table 2).

Biological sampling

We sampled demersal fishes using a 3/4 Yankee bottom trawl (11.9 m headrope, 15.5 m footrope, and 2.2 m wing lines) with 89 and 64 mm trammel mesh and 13 mm cod end mesh (all mesh sizes were stretch measure). Bottom trawls were fished for 30 min tows at each station at 3–4 km·h^{-1}. Bottom trawl wingspread data were recorded at roughly 10 s intervals using a NETMIND net mensuration

Table 1. Contrast in basic attributes between kiyi (*Coregonus kiyi*) and adult cisco (*Coregonus artedi*) suggesting different expectations for DVM behavior.

	Adult cisco	Kiyi	Source
Maximum size	495 mm	300 mm	Bersamin 1958; Smith 1964; Stockwell et al. 2006; Negus et al. 2008
Siscowet predation	Size refuge	Vulnerable	Damsgård 1995; Keeley and Grant 2001
Diet	Copepods and cladocerans	*Mysis relicta*	Anderson and Smith 1971; Johnson et al. 2004
Demersal behavior	No	Yes	Stockwell et al. 2006; Yule et al. 2007

Fig. 1. Location of eight stations sampled in Lake Superior in 2005 and 2006. See Table 2 for sampling details.

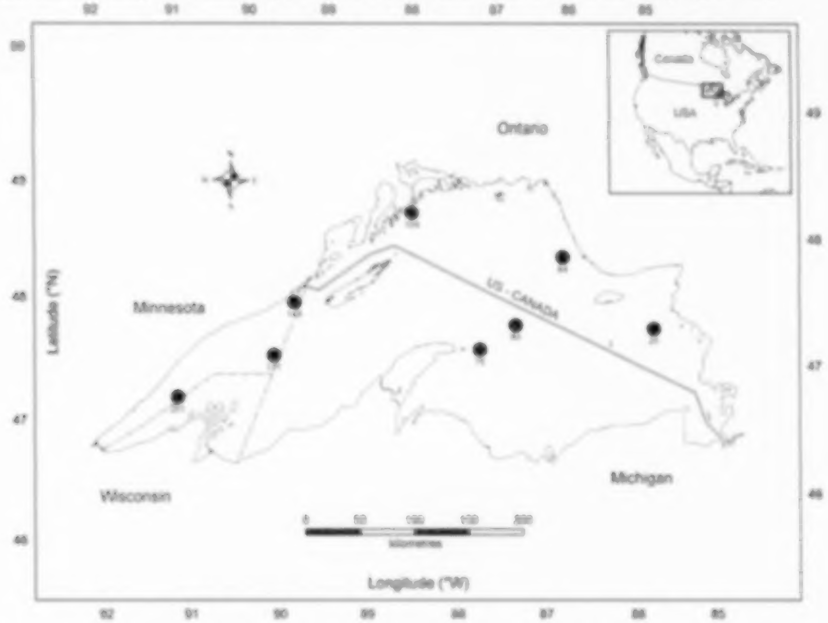


Table 2. Stations, bathymetric depths, and frequencies (kHz) used to sample fish density, size, and depth distributions with acoustics.

			Spring		Summer		Fall	
Year	Station	Bathymetric depth (m)	Day (kHz)	Night (kHz)	Day (kHz)	Night (kHz)	Day (kHz)	Night (kHz)
2005	25	297	120[a]	120 (2)	N/A	70 (1)	N/A	120 (1)
2005	76	168	120	120 (2)	70	70 (2)	120	120 (2)
2005	80	327	120[a]	120 (2)	N/A	70 (2)	N/A	N/A
2005	84	180	120	120 (2)	N/A	70 (2)	N/A	120 (2)
2005	106	147	120	120 (2)	70	70 (2)	N/A	120 (1)
2005	149	245	120	120 (2)	N/A	N/A	N/A	120 (2)
2005	171	160	120	120 (2)	N/A	70 (2)	120	120 (2)
2005	201	191	120	120 (2)	N/A	N/A	N/A	120 (2)
2006	171	160	120	120 (2)	120	120 (2)	120	120 (2)
2006	201	191	120	120 (2)	120	120 (2)	120	120 (2)

Note: The two acoustic units were both DT-X digital echosounders (Biosonics, Inc., Seattle, Washington) with either a 120 kHz split-beam transducer (beam width = 6.7°) or a 70 kHz split-beam transducer (beam width = 5.3°). Numbers in parentheses in the Night columns indicate the number of replicates used to estimate fish density, size, and depth distributions at night with acoustics. For sampling events with two replicates, the average of the two replicates was used. Only one replicate was collected during day sampling. N/A, not available.

[a] Not included in daytime analyses because station depth exceeded the range of acoustic gear (see Materials and methods).

system (Northstar Technical, Inc., St. John's, Newfoundland).

We sampled pelagic fish at night using a midwater trawl with 15.2 m headrope and footrope lines and 13.7 m breast lines. The mesh tapered from a stretch measure of 152 mm at the mouth to 13 mm at the cod end. Midwater trawl tows were conducted in a stepped-oblique fashion (Kirn and LaBar 1991) at 4–5 km·h^{-1} over the same transect path of bottom trawl tows. The trawl was fished starting at 5 m headrope depth and stepped down in 10–15 m increments to a maximum headrope depth of 80–90 m, with approximately equal fishing time at each depth. Few acoustic targets were detected below 100 m at night at all sites. Midwater trawl tow durations (range, 35–80 min; median, 54 min) were greater than those for bottom trawl tows to increase fish sample sizes in midwater trawls. Midwater trawls were not fished during the day. Previous attempts were not successful, suggesting that fish evade the trawl during this time (Yule et al. 2007).

All trawl catches were sorted by species, enumerated, and weighed in aggregate to the nearest gram. We measured total length to nearest millimetre for individuals of each species. When catches were large (>200 individuals for a species), we randomly selected 50–100 individuals to measure total length. We estimated density for each species captured in bottom trawl tows by dividing numbers caught by area swept (distance on bottom multiplied by average wingspread).

Acoustic sampling

Acoustic data to measure fish densities, acoustic sizes, and depth distributions were collected concurrently with all bottom and midwater trawl samples. For most sampling, we used a DT-X digital echosounder (Biosonics, Inc., Seattle, Washington) equipped with a 120 kHz split-beam circular transducer with a half-power beam width of 6.7° (Table 2). The transducer was mounted on a 1.2 m tow body and deployed approximately 1 m below the surface on the port side of the R/V *Kiyi*. During the summer of 2005 when our 120 kHz sounder failed (Table 1), we used a Biosonics DT-X digital echosounder equipped with a 70 kHz split-beam circular transducer with a half-power beam width of 5.3°. Previous studies (Rudstam et al. 1999; Mason and Schaner 2001; Godlewska et al. 2009) have shown that these two frequencies provide comparable data.

Acoustic signals were collected with BioSonics Visual Acquisition Software (version 4.1), and output files were stored to a laptop computer hard drive. Vessel position was measured with an Ashtech BRG2 (Ashtech Corp., Santa Clara, California) differentially corrected global positioning system (GPS) unit (accurate to 1 m), and this information was embedded in the acoustic data files. A transmit pulse duration of 0.4 ms was used at all times. Sampling rate was set at 3 pings·s^{-1} when transect maximum depths were less than 150 m. Stations with bathymetric depths greater than 150 m were sampled at 1–2 pings·s^{-1}. This approach maximized the amount of acoustic information collected while eliminating the problem of acoustic "cross talk" that occurs when one ping is not allowed to return to the transducer before a subsequent ping is transmitted. At the two deepest stations, we set the maximum range at 248 m — the maximum range at which the DT-X system could collect data in split-beam mode. Thresholds were set to allow detection of all echoes exceeding –75 dB on the acoustic axis.

The acoustic systems were calibrated periodically in the field using a 33 mm calibration sphere with an expected –40.5 dB (dB) target strength (TS) using techniques recommended by BioSonics. Results of these field tests indicated agreement with laboratory calibrations and consistent sensitivity throughout the study.

Acoustic data were processed using Echoview software (version 3.45.54.2627, SonarData Pty Ltd., Tasmania, Australia). Regions of echograms containing non-fish echoes (e.g., noise from electrical interference) were excluded from analyses. A line was drawn in each echogram at approximately 0.5 m above the lake bottom to exclude bottom echoes from analysis. The water column was analyzed in 5 m depth layers for the full length of each transect. We applied a minimum threshold of –65 dB before measuring the average volume backscattering of fish echoes in each 5 m layer.

Single targets were grouped into two TS size classes: small prey ($-55 < \text{TS} < -35.6$ dB) and large prey ($-35.6 \le \text{TS} < -32$ dB). Yule et al. (2006, 2009) demonstrated that large cisco (>250 mm total length) could be separated effectively from other pelagic prey species in Lake Superior using a –35.6 dB breakpoint. For each layer, the proportion of all single targets occurring in each TS size class was used to allocate total volume backscattering to the two TS size classes. The mean TS for each prey size class was then used to calculate density (fish·ha^{-1}) in each 5 m layer by scaling the proportional echo backscattering. Backscatter from predatory fish ($\text{TS} > -32$ dB; Hrabik et al. 2006) was subtracted from the total backscatter prior to density estimation of prey fish. Total areal densities of each size class were calculated by summing fish densities of all of the 5 m vertical layers. To understand how fish depth distributions were related to temperature, we collected a thermal profile using a SEACAT Model 19 Profiler (Sea-Bird Electronics, Inc., Bellevue, Washington) at each station during each sampling event.

To estimate pelagic prey fish species densities at night for a given station, we assigned captured fish from the corresponding midwater trawl to two size classes (small, < 250 mm; large, ≥250 mm). We summed the number of small and large fish captured in each midwater trawl and calculated the proportion of each species in each size class. We then multiplied the acoustic density estimate of small and large prey fish by the species proportions of each size class to estimate densities of each species. In some instances, large fish (i.e., >250 mm) were not captured in a midwater trawl despite an acoustic density estimate greater than 0 for large fish. In these cases, we assumed that these large fish were cisco. Densities of pelagic siscowet were not calculated because of their near absence in night midwater trawl samples. Siscowet densities at each station were estimated from bottom trawl samples only.

Statistical analyses

To test for DVM behavior, we compared day and night density estimates from bottom trawl tows for each species using a paired *t* test. Based on observations from Hrabik et al. (2006), we expected day bottom trawl densities to be higher than night densities for each species. We therefore used a one-tailed test ($\alpha = 0.05$).

Because of the preponderance of siscowet and kiyi in day bottom trawl tows, we compared density estimates of the two species from day bottom trawl tows using Pearson's correlation coefficient r. We performed two tests: the first used density estimates of all sizes of siscowet and the second used density estimates of siscowet exceeding 478 mm. We used densities of siscowet exceeding 478 mm to correspond to the sizes of predatory siscowet defined in the Hrabik et al. (2006) study. Our significance level was set at $\alpha = 0.05$, but we adjusted α using a Bonferroni correction for the two tests (i.e., $\alpha = 0.05/2 = 0.025$).

To test hypotheses regarding the magnitude of DVM of prey species, we estimated the median depths occupied by kiyi and cisco on each sampling event. We used density estimates from both pelagic (acoustic) and demersal (bottom trawl) habitats for these calculations. For most night sampling events, we had two replicates of acoustic data (one for bottom trawl and one for midwater trawl sampling; see Table 2), so depth-specific densities were estimated by averaging these replicates. Estimating median depths from night samples was straightforward because we could assign pelagic density estimates to species based on midwater trawl catches. For day pelagic estimates, we did not have any midwater trawl samples, so we assumed that all small prey (<–35.6 dB) were kiyi and all large prey (–32 dB > targets > –35.6 dB) were cisco. We calculated the magnitude of DVM as the difference between day and night median depths.

We used a paired t test to examine the difference in the magnitude of DVM between kiyi and cisco. We expected kiyi to exhibit a greater migration so we used a one-tailed test ($\alpha = 0.05$). We used linear regression to test for an effect of siscowet density on magnitude of DVM for kiyi and cisco. Siscowet density was calculated from day bottom trawl samples. We performed two tests using density estimates for all sizes of siscowet and for large siscowet (≥478 mm) separately. Our significance level was set at $\alpha = 0.05$, which we adjusted using a Bonferroni correction for the two tests (i.e., $\alpha = 0.05/2 = 0.025$).

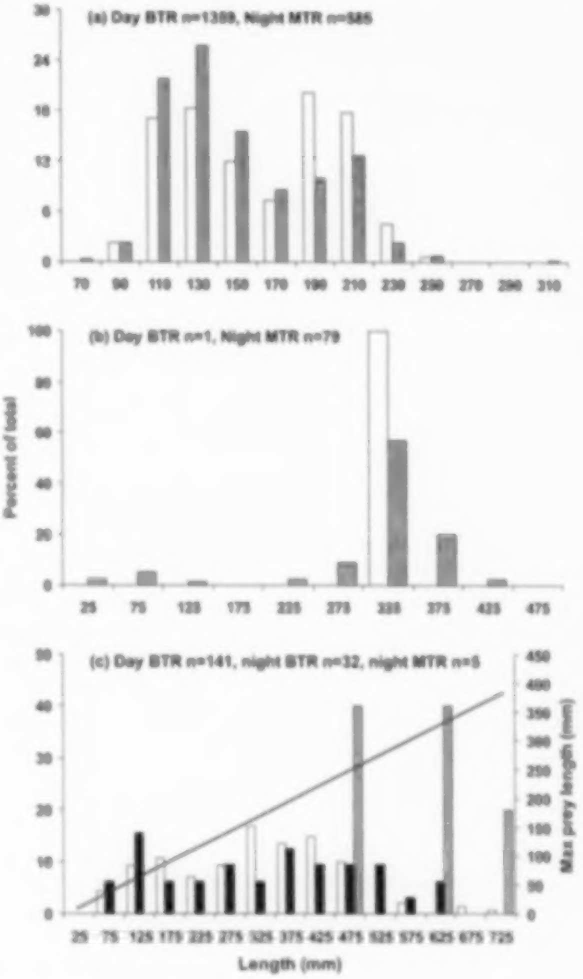
Results

Species composition and size distributions

We caught an estimated 30 753 fish in all trawls at the eight offshore stations. Four species (deepwater sculpin, kiyi, siscowet, and cisco) represented 99.5% of the total catch. Deepwater sculpin was the most numerous (12 190 individuals) species in day bottom trawls, followed by kiyi (3141) and siscowet (141). Deepwater sculpin was also the most numerous (14 368) in night bottom trawls, followed by siscowet (32), kiyi (13), and slimy sculpin (*Cottus cognatus*; 13). Kiyi was the most numerous (640) species captured in night midwater trawls, followed by cisco (79) and rainbow smelt (*Osmerus mordax*; 33).

Collectively, the two predominant coregonid species had three distinct size modes. The length–frequency distribution for kiyi was bimodal, with modes at 130 mm and 190–210 mm (Fig. 2*a*), whereas most cisco were large (mode = 325 mm; Fig. 2*b*). Only one cisco was captured with day bottom trawl tows.

Fig. 2. Length–frequency distributions of (*a*) kiyi (*Coregonus kiyi*), (*b*) cisco (*Coregonus artedi*), and (*c*) siscowet (*Salvelinus namaycush*) captured in day bottom trawl (BTR) samples (open bars), night BTR samples (solid bars), and night midwater (MTR) trawl samples (shaded bars) in 2005 and 2006. Sample sizes (n) are listed in each panel. The equation for maximum (Max) prey length in (*c*) is prey length (cm) = 0.535 × predator length (cm) – 0.487 (Damsgård 1995).



Siscowet captured in both day and night bottom trawl tows had a broad length–frequency distribution, with lengths ranging from 50 mm to >750 mm (Fig. 2*c*) with no prominent modes. Only five siscowet were captured in midwater trawl tows at night, but these fish were generally larger (range 450–750 mm) than siscowet captured in bottom trawl tows (Fig. 2*c*).

Based on the maximum predicted prey size equation for brown trout (*Salmo salar*) developed by Damsgård (1995), we calculated that only 7% of all siscowet captured were of sufficient size (>550 mm) to consume a prey fish larger than 300 mm (Fig. 2*c*). In contrast, 46% of all siscowet captured were of sufficient size (>380 mm) to consume a prey fish smaller than 200 mm. For coregonids, 80% of all cisco captured exceeded 300 mm (Fig. 2*b*), whereas 80% of all kiyi captured were less than 200 mm (Fig. 2*a*).

Spatial coherence between siscowet and kiyi

There was a significant positive correlation between density of kiyi and siscowet (all sizes) in day bottom trawl tows ($r = 0.80$, $p < 0.0001$, $n = 19$; Fig. 3*a*), suggesting that siscowet maintain a spatial association with kiyi. The correlation was positive ($r = 0.44$) but not quite significant ($p = 0.06$) between large siscowet (≥478 mm) and kiyi (Fig. 3*b*).

Diel vertical migration

Data for the two dominant coregonid species indicate differing DVM patterns. Kiyi appear to undergo very strong DVM, with much of their population on or near the bottom during daylight hours. Mean density (± standard deviation, SD) from day bottom trawl tows across 18 station/season samples was 76.0 ± 95.2 fish·ha^{-1} compared with 0.4 ± 0.8 fish·ha^{-1} from night bottom trawl tows (mean difference = 75.6 fish·ha^{-1}, SD = 94.7, t ratio = 3.39, $p = 0.0017$, df = 17; Fig. 4*a*). Pelagic density estimates of small prey fish (<–35.6 dB; presumably kiyi) during the day averaged 13.9 ± 9.8 fish·ha^{-1}, or 18% of the mean density of kiyi from day bottom trawl tows. Night pelagic density estimates for kiyi ranged from 12 to 295 fish·ha^{-1}, with a mean density of 111.0 ± 77.8 fish·ha^{-1}.

The magnitude of cisco DVM appears smaller than kiyi. Only one cisco was captured with day bottom trawl tows, and none was captured with night bottom trawl tows, suggesting that this species was pelagic during both photoperiods. Pelagic density estimates for cisco at night using acoustics and midwater trawls ranged from <1 to 29 fish·ha^{-1} and averaged 5.4 ± 5.8 fish·ha^{-1}. Pelagic density estimates of large prey fish during the day (≥–35.6 dB; presumably cisco) ranged from 0.1 to 1.6 fish·ha^{-1} and averaged 0.7 ± 0.6 fish·ha^{-1}.

Changes in density estimates of siscowet between day and night bottom trawl tows suggest that most siscowet perform DVM (Fig. 4*b*). Mean density (±SD) from day bottom trawl tows across the 18 sampling events was 4.2 ± 3.4 fish·ha^{-1} compared with 1.0 ± 0.9 fish·ha^{-1} from night bottom trawl tows (mean difference = 3.1 fish·ha^{-1}, SD = 3.3, t ratio = 4.08, $p = 0.0004$, df = 17). Density estimates of siscowet ≥ 478 mm in our day and night bottom trawl tows averaged 0.4 ± 0.5 fish·ha^{-1} and 0.2 ± 0.5 fish·ha^{-1}, respectively, but these were not significantly different (mean difference = 0.2 fish·ha^{-1}, SD = 0.7, t ratio = 0.92, $p = 0.18$, df = 17).

Kiyi were concentrated primarily on the bottom during the day, regardless of season, but were found in distinct depth layers at night, which varied by season (Fig. 5). Night distributions peaked at 65 m in the spring (Fig. 5*a*), 12.5 m in summer (Fig. 5*b*), and 17.5 m in the fall (Fig. 5*c*). Season explained 96% of the variation in median depth of kiyi at night (F ratio = 289.1, $p < 0.0001$, df = 26). Thermal profiles indicated isothermal conditions in the spring, strong stratification in the summer, and weakening of the thermocline in fall (Fig. 5).

Day distributions of cisco were much more variable than kiyi and appeared to be split between shallower and deeper pelagic depths during all three seasons (Fig. 5). Season explained a significant amount of variation ($R^2 = 0.39$) in the median night depth distribution of cisco (F ratio = 7.75, $p < 0.003$, df = 26), but much less than the variation explained for kiyi ($R^2 = 0.96$).

Fig. 3. Bivariate plot of siscowet (*Salvelinus namaycush*) ((*a*) all sizes and (*b*) ≥478 mm) and kiyi (*Coregonus kiyi*) densities from day bottom trawl (BTR) tows.

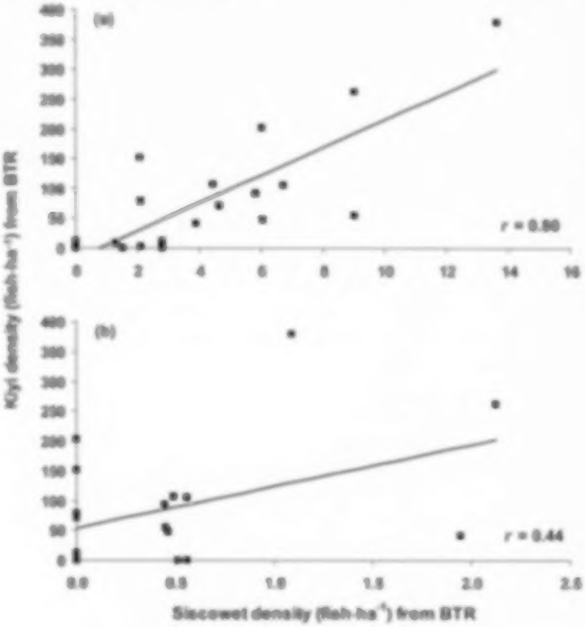


Fig. 4. Paired day and night bottom trawl (BTR) density estimates of (*a*) Lake Superior kiyi (*Coregonus kiyi*) and (*b*) siscowet (*Salvelinus namaycush*). Line represents unity in each panel.

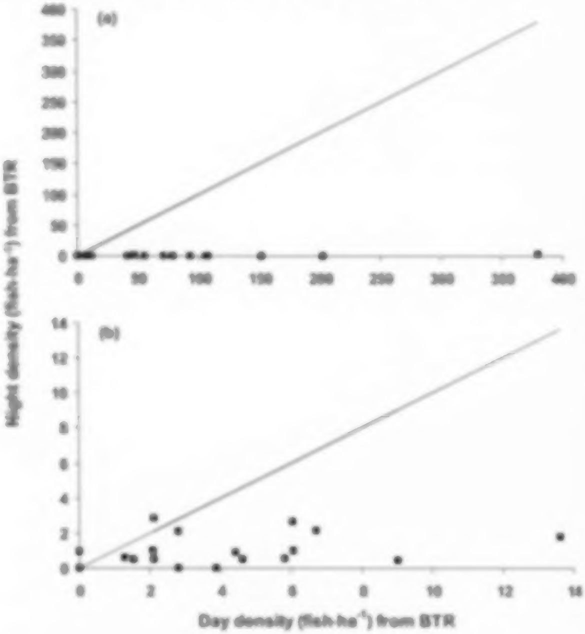
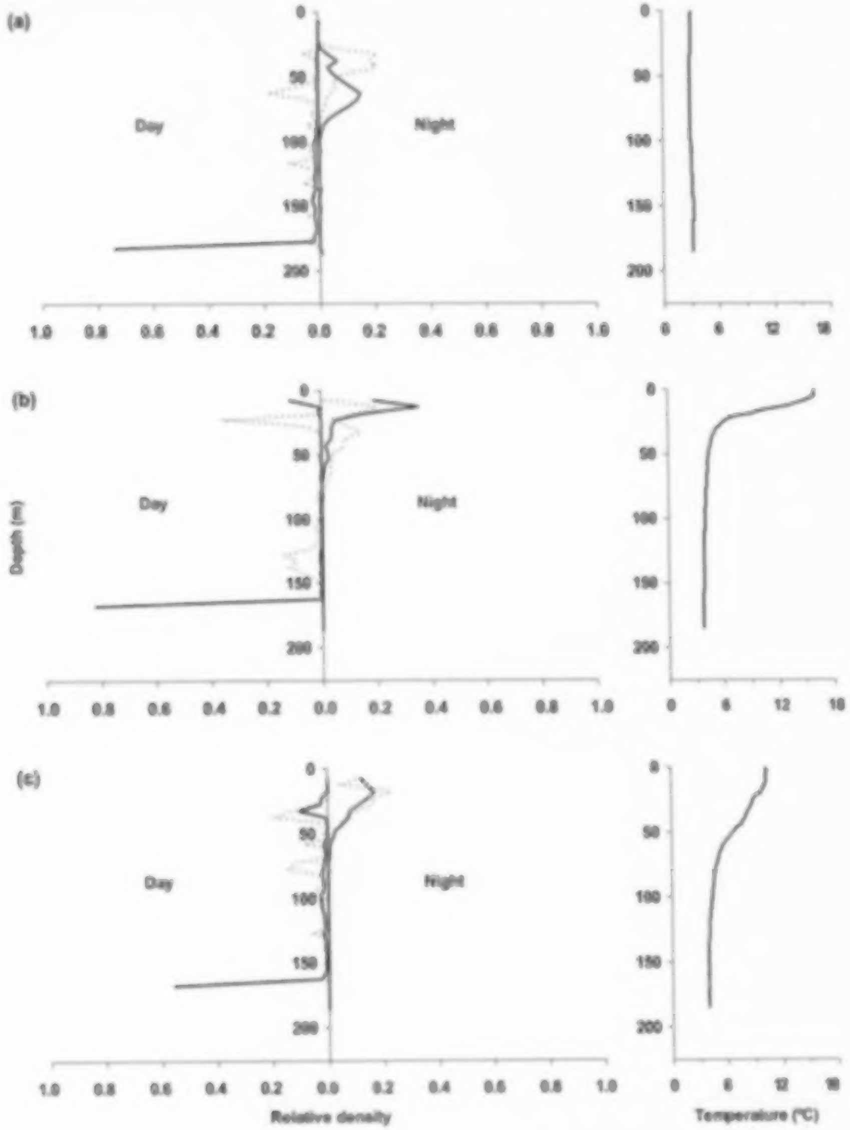


Fig. 5. Mean relative density of kiyi (*Coregonus kiyi*) (continuous line) and cisco (*Coregonus artedi*) (broken line) as a function of depth in Lake Superior during day and night sampling in (*a*) spring, (*b*) summer, and (*c*) fall of 2005 and 2006. Mean thermal profiles for each season are also shown.

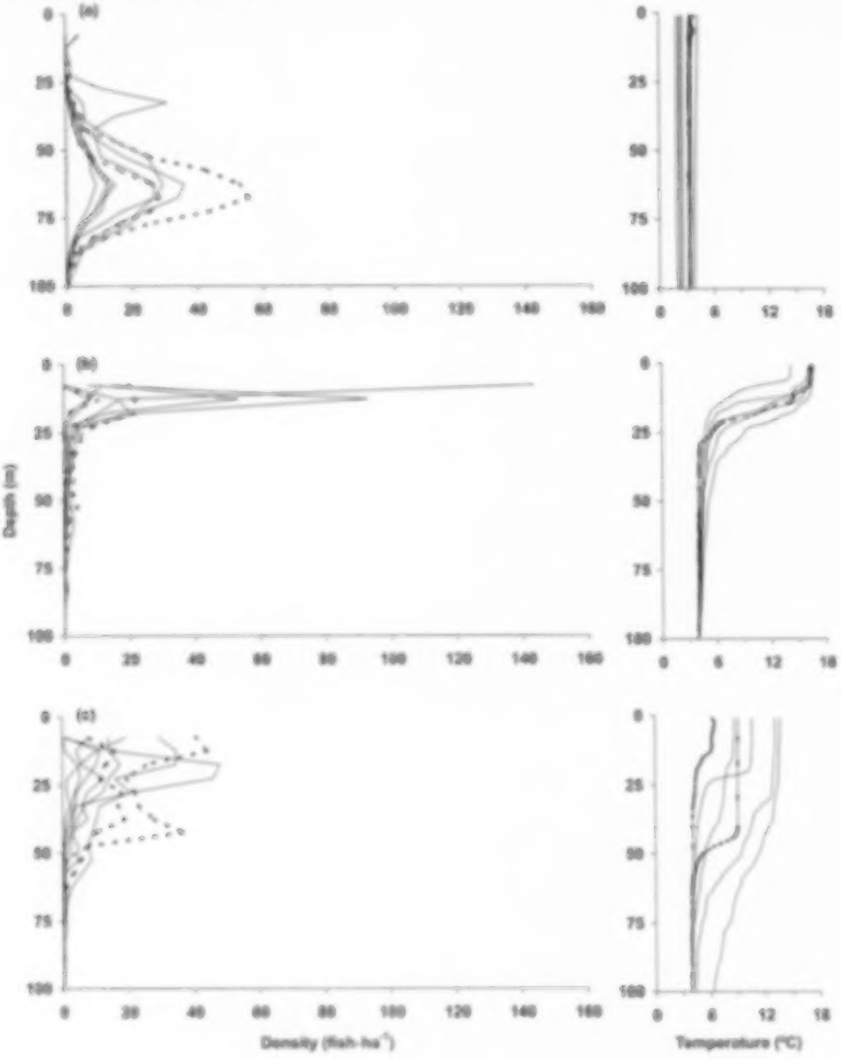


When kiyi and cisco DVM trajectories were compared, several patterns emerged. First, kiyi were much deeper than cisco during the day (Fig. 5). Second, kiyi were mostly demersal during the day. Third, the general pattern in night distributions changed across seasons. Kiyi were deeper than cisco in spring, shallower in summer, and similar in fall (Fig. 5). Finally, night depth distributions of kiyi were remarkably consistent across stations within each season (Fig. 6), whereas cisco exhibited more variability (Fig. 5). Kiyi performed significantly greater DVM than cisco (mean difference in DVM = 63 m, SD = 52.4, t ratio = 4.82, $p < 0.0001$, df = 15; Fig. 7). The magnitudes of DVM for kiyi and cisco were not related to density of all siscowet (for kiyi, $p = 0.67$, $R^2 = 0.01$; for cisco, $p = 0.30$, $R^2 = 0.08$; Fig. 8*a*) or siscowet ≥ 478 mm (for kiyi, $p = 0.60$, $R^2 = 0.02$; for cisco, $p = 0.72$, $R^2 = 0.01$; Fig. 8*b*) as measured by day bottom trawl samples.

Discussion

One prediction of the antipredation window hypothesis is that DVM behavior is a size-dependent process. That is, as an individual grows and begins to attain a size refuge from predators, the relative importance of potential predation risk in the risk versus growth trade-off should decrease. Within

Fig. 6. Night depth distribution of kiyi (*Coregonus kiyi*) and thermal profiles of Lake Superior in (*a*) spring, (*b*) summer, and (*c*) fall of 2005 (continuous lines) and 2006 (broken lines). Each profile represents an individual site. The depth axis is truncated at 100 m because very few fish were observed below this depth at night.



the framework of Jensen et al. (2006), we would expect larger-bodied cisco to follow a shallower DVM trajectory compared with kiyi. Although differences that we observed in this study are consistent with this prediction, known differences in the ecology of kiyi and adult cisco, the demersal co-occurrence of kiyi and siscowet during the day, and the apparent size refuge of adult cisco from siscowet predation suggest that predator avoidance is not a proximate factor responsible for their divergent DVM behaviors. We compare and contrast the DVM behavior of kiyi and adult cisco in the context of potential predation risk, foraging opportunities and bioenergetic efficiencies, and food web dynamics.

Potential predation risk

Our results show that the extent of adult cisco DVM is of lesser magnitude than that of kiyi. Cisco are located higher in the water column during the day, an area where the reaction distance of visually foraging siscowet is high (Jensen et al. 2006). However, Harvey and Kitchell (2000) and Harvey et al. (2003) provided isotopic evidence that cisco are not key components of siscowet diet in Lake Superior where depths exceed 80 m. We found that 80% of the cisco in offshore waters exceeded 300 mm. Using the Damsgård (1995) model to predict maximum prey size, we estimated that a 300 mm cisco would be vulnerable to only 7% of the siscowet that we captured. Keeley and Grant (2001) provide evidence that Damsgård's (1995) model provides reasonable predictions for piscivorous lake trout. Recent work on Lake Superior has shown that adult cisco have high annual survival rates (>0.75; Yule et al. 2008) and that cisco are a mi-

Fig. 7. A comparison of the magnitude of diel vertical migration (DVM) for kiyi (*Coregonus kiyi*) and cisco (*Coregonus artedi*) in spring (solid circles), summer (open squares), and fall (solid triangles). Line represents unity.

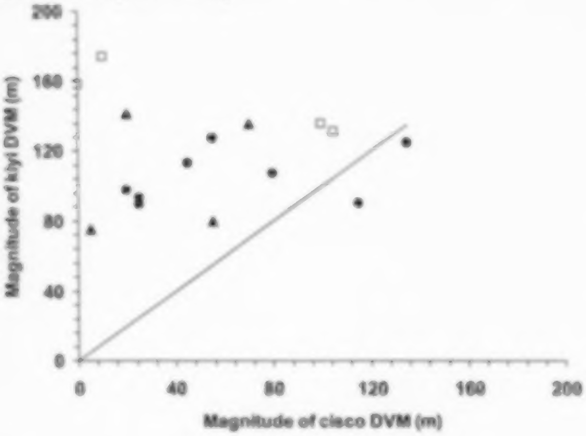
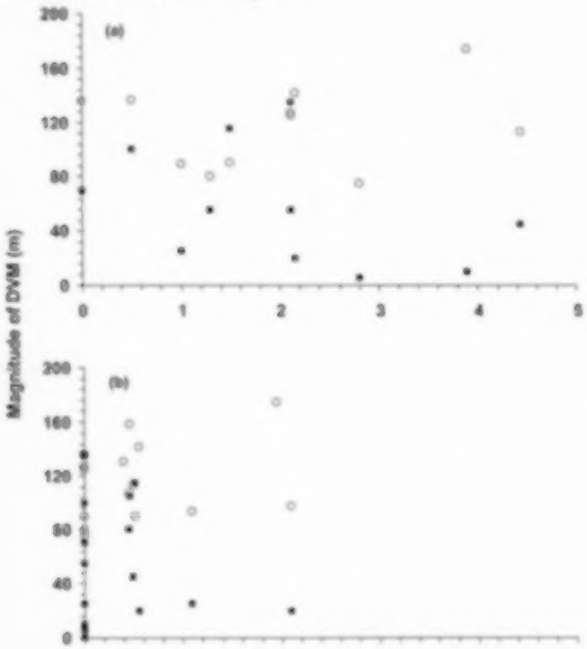


Fig. 8. Relationship between magnitude of diel vertical migration (DVM) of kiyi (*Coregonus kiyi*) (open circles) and cisco (*Coregonus artedi*) (solid squares) and density estimates of (*a*) all siscowet (*Salvelinus namaycush*) and (*b*) large (>478 mm) siscowet from day bottom trawl (BTR) samples.



nor component of the siscowet diet (Negus et al. 2008). Both studies suggest that cisco have a functional size refuge from most siscowet in offshore waters of Lake Superior. Without strong constraints imposed by predators, they should exhibit shallower and more variable daytime depth distributions (Jensen et al. 2006). Our findings support this prediction.

If adult cisco are largely invulnerable to siscowet predation, why do they conduct DVM? Negus et al. (2008) reported that "lean" lake trout (the shallow-water morphotype of lake trout) were the dominant predator in nearshore (<80 m bathymetric depths) waters of Wisconsin and Minnesota, with coregonids (mostly cisco) representing approximately 60% of their diet by weight. Cisco are widely distributed in Lake Superior, with younger cisco smaller than 250 mm generally occupying nearshore waters and adults occupying the entire lake (Stockwell et al. 2006). Although cisco have an apparent size refuge in offshore waters, predation pressure on cisco in nearshore waters appears to be greater (Negus et al. 2008). We hypothesize that cisco DVM behavior in offshore waters may be a residual effect of managing potential predation risk from lean lake trout in nearshore waters.

Unlike cisco, a large proportion of the kiyi that we sampled were likely vulnerable to predation by siscowet, which is consistent with recent siscowet diet data from Negus et al. (2008). Kiyi exhibited greater DVM than adult cisco and were consistently deeper during the day. This observation indicates more risk-averse behavior for kiyi and follows expectations of the antipredation window hypothesis. However, at the spatial and temporal scale of this study, we found no evidence that kiyi (or cisco) alter the extent of their DVM in response to differences in predator densities. Moreover, the co-occurrence of kiyi and siscowet at great demersal depths during the day, where the lack of light most likely precludes the use of vision for predation by either siscowet (on kiyi) or kiyi (on *M. relicta*), indicates that light is not likely a proximate factor for daytime distributions of siscowet or kiyi and that predator avoidance is not an ultimate factor for daytime distributions of kiyi.

Foraging opportunities and bioenergetic efficiency

Although cisco feed to a small extent on the macroinvertebrate *M. relicta*, copepods and cladocerans are the primary prey of cisco in Lake Superior (Dryer and Beil 1964; Anderson and Smith 1971; Johnson et al. 2004). These smaller plankters generally occupy upper parts of the water column and perform limited DVM (Conway 1977; Zhou et al. 2001; Yurista et al. 2009). An alternative to the hypothesis that adult cisco perform DVM in the offshore waters as a residual effect from potential predation risk in nearshore waters by lean lake trout is that cisco perform DVM to optimize their energetic efficiency. This alternative hypothesis could be tested using the framework of Jensen et al. (2006), modified for the species-specific characteristics of adult cisco.

In contrast to the diet of adult cisco, *M. relicta* is the primary prey of kiyi (Anderson and Smith 1971; A. Gamble, University of Minnesota, Department of Biology, Duluth, Minnesota, unpublished data) and undergoes extensive DVM in Lake Superior (Bowers 1988; Jensen et al. 2009). We observed that the vertical distributions of kiyi at night varied across seasons but showed remarkable consistency across stations separated by 100s of kilometres within each season. During the day, kiyi were primarily bottom-oriented in all seasons. In spring when the water column was isothermic, nighttime distributions of kiyi were deeper than in summer or fall. In summer and fall, kiyi ascended nearer to the surface at night and appeared to relate to thermal structure in these depth zones. Temperature and light are primary drivers of *M. relicta* DVM and explain the deeper depth distributions of this macroinvertebrate in Lake Ontario in the spring during isothermic conditions and their proximity to the thermocline in summer and fall (Gal et al. 2004; Boscarino et al. 2007). If *M. relicta* in Lake Superior follow similar patterns, it might explain the deeper night distributions of kiyi during the spring and their association with the thermocline in summer and fall. Such relationships between nighttime distributions, invertebrate prey, and temperature across seasons have also been observed for vendace (*Coregonus albula*) and Fontane cisco (*Coregonus fontanae*), suggesting that thermal stratification may play an important role in the magnitude of DVM and nighttime depths of coregonids (Helland et al. 2007; Mehner et al. 2007; Busch and Mehner 2009).

Eshenroder and Burnham-Curtis (1999) hypothesized that deepwater ciscoes in the Great Lakes, including kiyi, evolved to feed on vertically migrating *M. relicta*. Kiyi likely underwent adaptive radiation from colonizing cisco (i.e., *C. artedi*) after the last glacial retreat 11 000 years ago (Smith and Todd 1984; Todd and Smith 1992). The species had to overcome a number of physical and biological challenges, including the effects of pressure changes when performing DVM, colder temperatures associated with a largely hypolimnetic existence, and possible foraging on *M. relicta* at great depths with presumably no light. Little, if any, research has been conducted on these facets of kiyi ecology. For example, comparison of gas bladder anatomy and lipid levels of cisco and bloater with kiyi would address ongoing questions of buoyancy regulation in coregonids (TeWinkel and Fleischer 1998; Eshenroder and Burnham-Curtis 1999; Clemens and Don Stevens 2007). Species-specific differences in thermal performance functions (e.g., swimming, standard and active metabolism) in sympatric coregonids suggest species adaptation to different temperature regimes (Helland et al. 2007; Ohlberger et al. 2008*a*, 2008*b*). By extension, energetic costs to kiyi and cisco performing DVM under similar environmental conditions (e.g., in a modeling context) are likely different. Similarly, foraging abilities and environmental constraints of each species likely differ. For example, large differences in eye size between kiyi and cisco suggest that kiyi may have greater visual acuity under low light conditions and presence of *M. relicta* in kiyi captured on bottom during the day suggests that kiyi are capable of nonvisual predation (A. Gamble, University of Minnesota, Department of Biology, Duluth, Minnesota, unpublished data).

Collectively, these thermal and physiological considerations strongly suggest that kiyi and adult cisco experience different growth (forage) rate potentials as a function of vertical depth, photoperiod (i.e., light), and prey fields. Development of bioenergetics-based foraging models reflective of each species' ecology and estimates of fine-scale spatiotemporal densities of *M. relicta*, especially in demersal habitats, would greatly improve modeling efforts to evaluate kiyi and cisco foraging and growth rate potential.

Food web dynamics

Strong horizontal correlation between predator and prey may occur at a variety of spatial and temporal scales (e.g., Rose and Leggett 1990; Benoit-Bird and Au 2003). The strong positive correlation between siscowet and kiyi across stations suggests that siscowet exhibit spatial coherence with kiyi over large spatial scales. Alternatively, but less likely, is the possibility that the correlation was caused by both siscowet and kiyi responding to a common indirect factor such as *M. relicta*. Stable isotope and diet studies indicate that *M. relicta* is not a key diet component of siscowet (Conner et al. 1993; Harvey and Kitchell 2000; Negus et al. 2008). Thus, it seems most probable that siscowet are tracking kiyi, which are likely aggregating near patches of their preferred prey, *M. relicta*. Future research in this area may shed light on the mechanisms underlying this association.

Deepwater sculpin are common in diets of siscowet (e.g., Conner et al. 1993; Ray et al. 2007; Negus et al. 2008) and have relatively high densities in offshore waters of Lake Superior (mean density ± SD; 388 ± 373.4 fish·ha^{-1} during the day and 467 ± 465.4 fish·ha^{-1} at night in our bottom trawl samples). Given that visual foraging by siscowet at the bathymetric depths we sampled is unlikely (Jensen et al. 2006), we hypothesize that siscowet are able to use senses other than vision to forage in deepwater benthic habitats (i.e., their reaction distance > 0 at 0 lx; Mazur and Beauchamp 2003). Further, we hypothesize that the more sedentary deepwater sculpin is likely an easier target for siscowet in this habitat compared with the more mobile kiyi.

Uncertainties

Although we documented a significant decrease in siscowet density using bottom trawl samples between day and night, our midwater trawl catches at night caught very few siscowet (five) that were disproportionately larger than bottom trawl catches. These patterns suggest that smaller-sized siscowet may (*i*) evade the midwater trawl, (*ii*) vertically migrate to unsampled habitats at night (e.g., suspended below 100 m or in surface waters), or (*iii*) move into nearshore areas. We suspect the first explanation is unlikely because larger siscowet would presumably be able to avoid the trawl better than smaller siscowet. The second explanation appears feasible, although it is not clear what benefit these habitats would provide compared with occupying similar nighttime depths as kiyi. It is unlikely that siscowet are concentrated at depths greater than 100 m because we observed very few acoustic targets in these depth zones, consistent with Mason et al. (2005). The third explanation is possible as Harvey et al. (2003) suggested that smaller siscowet might rely on nearshore production to some degree, a finding substantiated by the diet work of Ray et al. (2007). This may be feasible for deepwater habitats relatively close to shore (e.g., stations 149 and 106) but unlikely for those further from shore (e.g., stations 171 and 80). Future work should consider shallow-set suspended gill nets in offshore waters or biotelemetry studies to better understand habitat usage of siscowet.

We assumed that all targets smaller than –35.6 dB in the water column during the day were kiyi. However, we captured some (albeit very few) smaller cisco and rainbow smelt in the same size range as kiyi (25–250 mm) in night midwater trawl tows, suggesting that smaller prey targets during the day may not have been (entirely) kiyi. Kiyi in the size range of 80–100 mm were captured in day bottom trawl tows, suggesting even these small fish undergo strong DVM. If smaller targets during the day were cisco, the distinct vertical distributions of large and small prey in summer in fall would be consistent with known ontogenetic thermal preferences within species (e.g., Viljanen 1983). Additionally, the bi- and multi-modal depth distributions of large prey targets during the day may also suggest size-dependent thermal preferences of adult cisco. However, given the narrow TS range that we defined for cisco (between −35.6 and −32 dB), such comparisons within this range would be meaningless. Milne et al. (2005) successfully captured cisco during daylight with suspended gillnets, so it is possible that this gear type could also lend itself to better understanding of daytime distributions of coregonids in Lake Superior.

In summary, we found that the two most effective DVM strategies modeled for a generalized coregonid in offshore waters of Lake Superior (Jensen et al. 2006) are utilized by two congenerics with different ecological niches. DVM patterns of kiyi and adult cisco were consistent with size-dependent predation risk as predicted by the antipredation window hypothesis. However, the apparent size refuge of adult cisco suggests that their potential predation risk is low regardless of their DVM trajectories, and the continual overlap of kiyi with siscowet day and night suggests that their potential predation risk is high despite an extensive DVM trajectory. The former nullifies the importance of the antipredation window for adult cisco, and the latter is contrary to expectations of the antipredation window hypothesis for kiyi. Both suggest that alternative factors play primary roles in DVM behavior. We suspect that because of their size refuge, adult cisco DVM in offshore waters likely optimize their bioenergetic efficiency. The consistency in the DVM pattern of kiyi with known DVM patterns of their primary prey *M. relicta* suggests that kiyi are vertically tracking their prey. The lack of a pelagic layer of kiyi during the day in the antipredation window may be a result of lack of suitable prey in that vertical layer; kiyi follow their prey to the bottom to realize some growth potential. Similarly, siscowet likely follow kiyi to the bottom where they also encounter an alternative prey source (deepwater sculpin) that they are known to consume rather than remain pelagic where appropriately sized prey appear to be scarce. In addition to prey tracking, ascent to the thermocline in summer and fall by kiyi, and presumably siscowet, may also increase gut evacuation rates and thus increase bioenergetic efficiency (Bevelhimer and Adams 1993).

Our study suggests that the factor(s) driving coregonid DVM may be more complex than first identified. It is apparent that the costs (potential predation risk) and benefits (foraging and growth) of DVM behavior differ drastically between kiyi and adult cisco. Light-based foraging theory is likely not applicable in the offshore demersal habitats of Lake Superior. Other factors need to be explored to better understand the extensive DVM of kiyi and siscowet. The challenge will be linking food web interactions between the two- (bottom) and three-dimensional (pelagic) habitats to account for alternative prey, shifts in foraging strategies and abilities, physiological performance in different thermal environments, and changes in potential predation risk that are likely present and driving the underlying differences in DVM exhibited by each coregonid species and siscowet.

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Survival and life history characteristics among wild and hatchery coho salmon (*Oncorhynchus kisutch*) returns: how do unfed fry differ from smolt releases?

Véronique Thériault, Gregory R. Moyer, and Michael A. Banks

Abstract: Survival and life history characteristics were evaluated for a coho salmon (*Oncorhynchus kisutch*) integrated hatchery program using two stocking strategies. Fish were released as unfed fry or smolts and returned as adults, and then molecular analysis was employed to pedigree the entire population. We showed that mean adult survival of individuals released as unfed fry was less than that of individuals released as smolts (0.03% vs. 2.39%). The relative reproductive success (RRS) of the fry release strategy to wild spawning was significantly greater for one of two cohorts, whereas the smolt release strategy to wild RRS was significantly greater for both cohorts. Fish released as smolts were significantly smaller upon returning as adults than either those released as unfed fry or wild returns. Mean run timing was also significantly biased towards an earlier run time for hatchery-released fish when compared with the wild component. The incidence of jacking (males maturing at age 2) was greater among fish stocked as smolts than for fish stocked as fry. Differences in survival, RRS, and life history appeared to be the result of hatchery practices and indicated that a fry stocking strategy produced fish more similar to the wild component of the population than to that of fish released as smolts.

Résumé : Nous avons déterminé la survie et les caractéristiques du cycle biologique de saumons coho (*Oncorhynchus kisutch*) dans un programme de pisciculture intégré qui utilise deux stratégies d'empoissonnement. Les poissons ont été ensemencés comme alevins ou saumoneaux à jeun et recapturés au stade adulte; des analyses moléculaires ont alors servi à établir la filiation de toute la population. Nous avons démontré que les poissons ensemencés au stade alevin à jeun avaient un taux de survie moyen jusqu'à l'âge adulte inférieur à celui des poissons ensemencés au stade saumoneau (0,03 % vs 2,39 %). Le succès reproducteur relatif (RRS) de la stratégie alevin par rapport aux individus en milieu naturel était supérieur pour une année, mais inférieur pour l'autre année. Le RRS de la stratégie saumoneau était supérieur pour les deux années. Les poissons ensemencés au stade saumoneau avaient une taille corporelle inférieure à l'âge adulte à celle des poissons relâchés en tant qu'alevins à jeun et des poissons sauvages. La date de montaison moyenne était significativement devancée pour les poissons d'élevage. L'incidence de la maturation sexuelle précoce (« jacking ») était plus grande parmi les poissons ensemencés au stade saumoneau que parmi les poissons relâchés au stade alevin. Ces différences dans la survie, le RRS et les traits d'histoire de vie semblent découler des pratiques d'élevage en captivité et indiquent que la stratégie d'ensemencement d'alevins produit des poissons qui sont plus semblables à l'âge adulte aux poissons sauvages que les ensemencements de saumoneaux.

[Traduit par la Rédaction]

Introduction

Worldwide declines in anadromous salmonid populations have led to the widespread use of hatchery propagation in efforts to boost population size and recover threatened or endangered populations. However, there are ecological and genetic risks associated with these substantial and sustained releases of hatchery fish (Ford et al. 2006; Kostow 2009). Integrated hatchery programs that obtain a proportion of brood stock from the local population (Goodman 2005) have been applied to mitigate potential negative genetic impacts of traditional hatchery practices (e.g., reduction of genetic diversity, local maladaptation, domestication selection, and outbreeding depression; reviewed by Araki et al. 2008; Hutchings and Fraser 2008). Despite the theoretical prediction that genetic risks should be lower in these state-of-the-art conservation hatchery programs, recent empirical evidence (Araki et al. 2007, 2008, 2009) has shown that as lit-

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tle as one generation in captivity is sufficient to generate differential reproductive success (RS) between hatchery and wild components. Although captive rearing may generate differences in important life history traits likely to affect reproductive success (Knudsen et al. 2006; Fritts et al. 2007), causal genetic and (or) environmental mechanism(s) responsible for these differences remain unclear.

Salmonid populations have been supplemented at various life stages (fertilized eggs to adults) in the past century. The choice of which life stage to stock depends on various tradeoffs between the species and systems considered, the hatchery capability, the likelihood and environmental costs of artificial selection, budgetary considerations, and the relative survival and return rate of fish released at different life stages (Letcher and Terrick 2001). In Oregon, USA, coho salmon (*Oncorhynchus kisutch*) were stocked exclusively as fry from 1890 until 1940. Improvements in hatchery practices and the finding that smolts (the life stage in which juvenile salmon first migrate from freshwater to marine environments) had a higher postrelease survival led to an increased number of smolt releases by the 1950s (Solazzi et al. 1999). Smolt releases are now the dominant stocking strategy used by most federal and state agencies for salmonids of the Pacific Northwest. However, fry releases still occur regularly in Oregon (Kostow 2009) because of the popularity of Oregon Department of Fish and Wildlife's (ODFW) Salmon and Trout Enhancement Program (STEP), which was implemented in 1981 as a means to engage the public in the restoration of salmonid species.

Despite decades of implementation, few attempts have been made to evaluate the efficiency of fry stocking programs, primarily because it is difficult to tag fry and track them through adulthood using conventional methods such as fin clipping or coded-wire tagging. Previous studies that evaluated the efficiency of unfed fry releases or presmolt releases (i.e., young-of-the-year juveniles fed in the hatchery for a few months before release) concluded that they were not effective at increasing either the rearing density of juvenile coho salmon or adult returns (McGie 1980; Nickelson et al. 1986; Solazzi et al. 1999). One caveat to these studies was the use of out-of-basin and multigenerational hatchery-origin stocks that could confound the limited success of stocking young life stages with poor performance of maladapted stocks (Hutchings and Fraser 2008). More recently, Caroffino et al. (2008) used a genetic parentage reconstruction approach and local-origin brood stock to conclude that hatchery steelhead (*Oncorhynchus mykiss*) whose offspring were released as unfed fry produced more age-2 juveniles than adults reproducing in the wild. Theoretically, if domestication selection that occurs during rearing reduces mean fitness of the hatchery population, then fish released at an earlier life stage (e.g., the fry stage) should be better adapted to the natural environment than releases of later life stages because there is little (if any) time for domestication selection to occur. There is anecdotal empirical evidence to support this hypothesis in Atlantic salmon (*Salmo salar*), where fish stocked as fry show greater survival from smolt to adulthood when compared with fish released as smolts (Rideout and Stolte 1988). No study has rigorously compared the fry versus smolt release strategies, thus very limited knowledge is currently available to assess whether unfed fry releases have different survival rates and life history characteristics than smolt releases.

The major objective of this study was to evaluate the relative efficiency of coho salmon released as unfed fry versus smolts for a conservation hatchery program (one that uses random 1:1 paired matings and equalizes parent contributions). By using microsatellite markers to assign returning adults to their most likely parents, we were able to (*i*) compare survival rates of fish returning to the river from which they were released as unfed fry or smolts, (*ii*) evaluate if releasing progeny of brood stock as unfed fry or smolts increased individual reproductive success (number of progeny that survived to reproduction) relative to those breeding in the wild, and (*iii*) compare size at age, run timing, and incidence of maturing at age 2 (i.e., jacking) for returning adults between coho salmon stocked as unfed fry and those stocked as smolts, as well as with the natural population.

Materials and methods

Sampling design

The North Umpqua River, Oregon, USA, is supplemented with hatchery coho salmon every year as a way of augmenting the recreational and commercial fisheries. This integrated hatchery program incorporates local brood stock by including natural fish (30%, non-adipose-fin-clipped) each year with their collection of hatchery fish (70%, adipose-fin-clipped), producing smolts that are used to supplement the natural-spawning population. Each smolt released from the hatchery is adipose-fin-clipped to identify it as a "hatchery" fish. In 2001, 2002, and 2003, ODFW collected 100 adipose-fin-clipped coho salmon of each sex (hereafter referred to as hatchery-reared, H), as well as 94, 100, and 102 non-adipose-fin-clipped coho salmon of each sex, respectively (hereafter referred to as wild-born, W; note that although W is designated as wild-born, W fish may be of hatchery ancestry because of the occasional mating of hatchery fish in the wild). The H brood stock was collected at Winchester Dam for the three years sampled (Fig. 1). In 2001, the W brood stock was also collected at Winchester Dam, whereas for 2002 and 2003, W fish were taken from Calapooya Creek at Nonpareil Dam (Fig. 1). Calapooya Creek is a tributary of the Umpqua River and has no previous history of supplementation; however, hatchery strays have been known to influence the system. In addition to the W fish taken as brood stock, we also have records of virtually all W fish passed above Nonpareil Dam for 2002 and 2003 that were allowed to spawn naturally (783 and 520 fish, respectively). These data are not available for 2001, as relevant sampling equipment was not installed that year. For each of the three years, males and females were randomly paired within each group ([H×H] and [W×W]) and spawned at ODFW's Rock Creek hatchery facility using single-pair mating (i.e., each male and female was used only once). The progeny from these crosses are referred to as F_1 hatchery fish. Eggs from each mating pair were incubated separately until the eyed-egg stage. At this point, family sizes were equalized by randomly sampling 140–150 eyed eggs per mating pair, which were then reared together to the smolt stage (for more details, see Moyer et al. 2007). The remaining eyed eggs were transferred to hatchboxes

Fig. 1. Map of the study area showing sampling sites of brood stock (Winchester and Nonpareil dams).

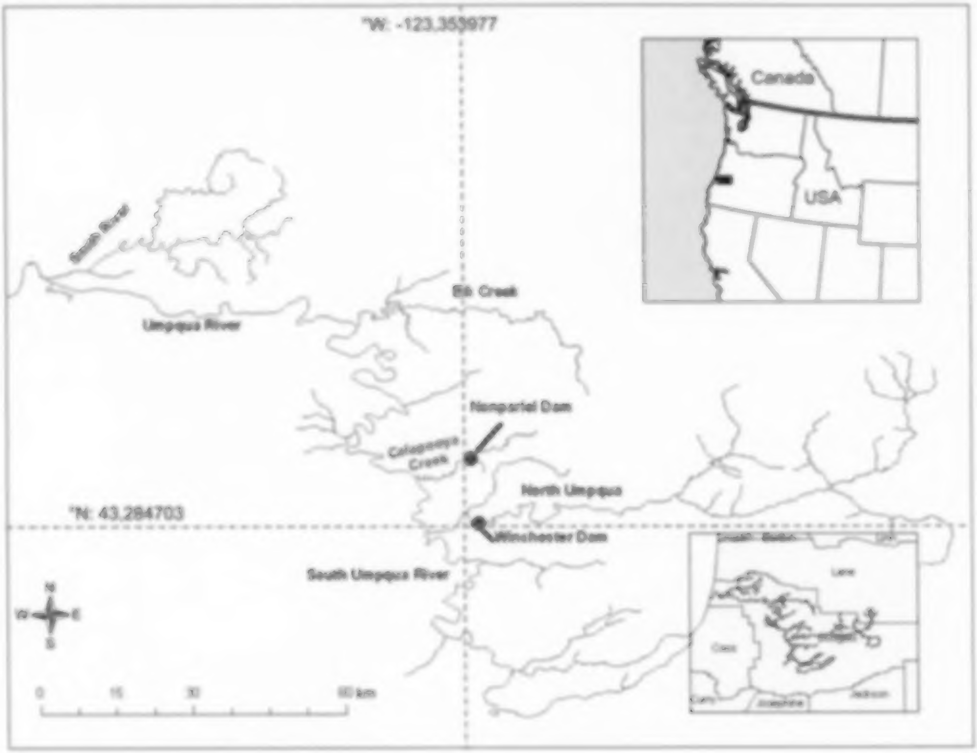


Table 1. Coho (*Oncorhynchus kisutch*) hatchery spawning and release information for the evaluation of survival in unfed fry and smolt release strategies for each brood year (BY).

		No. released		No. of returns		Survival (%)	
BY	No. of pairs spawned	Fry	Smolt	Fry	Smolt	Fry	Smolt
2001	194	370 576	24 373	79	360	0.02	1.48
2002	200	491 866	21 997	207	693	0.04	3.15
2003	202	445 628	24 372	398	622	0.09	2.55

Note: Numbers of returns as adults are given based on pedigree analysis, and survival rate is calculated as the number of fish that returned over the number released.

where three families were mixed to get a total of between 4000 and 6000 eggs and reared together in the same hatchbox for later release as unfed fry (see below).

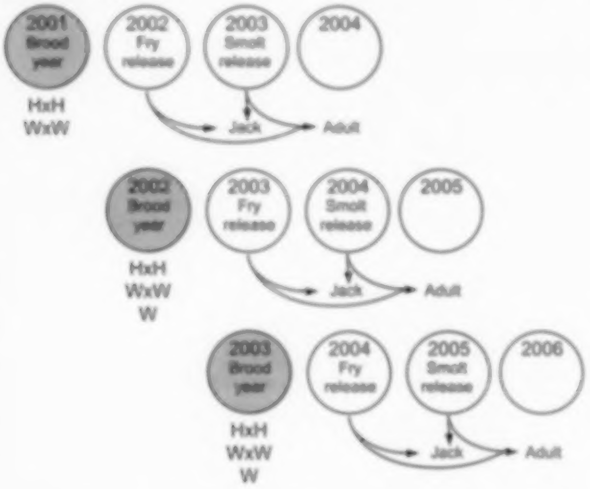
In spring 2002, 2003, and 2004, a few days after the absorption of the yolk sac, F_1 unfed fry were released in nine different sites along Calapooya Creek and two of its tributaries (Coon and Gassy creeks) above Nonpareil Dam (Fig. 1; Table 1). In spring 2003, 2004, and 2005, F_1 smolts of each brood stock were also released above Nonpareil Dam in Calapooya Creek (Fig. 1; Table 1). Smolts were clipped adipose left maxillary or adipose right maxillary to designate H×H or W×W origin, respectively. Unfed fry remained unmarked.

Most coho salmon migrate to the ocean after spending a year in freshwater. Some males spend only one summer at sea and return to freshwater in the following fall as mature males that are two years of age (termed jacks). The majority of males and all females spend two summers in the ocean before their migration to spawning habitat at three years of age. Therefore, fry and smolts released in 2002–2005 returned as jacks and adults in 2003–2006 (designed as return year (RY); Fig. 2). All returns to Calapooya Creek were captured, measured, sexed by visual identification, aged (only for 2- or 3-year-old males, based on size), and caudal-fin-clipped at a fish trap constructed at the base of Nonpareil Dam. All were released above the dam after handling.

Parentage analysis

The DNA from all brood stock and progeny returning to Calapooya Creek from 2002 to 2006 was extracted, amplified via polymerase chain reaction (PCR), and genotyped for 10 microsatellite loci (*OTS*519, *OTS*520, *ONE*111, *P*53,

Fig. 2. Study design showing the three brood years (shaded) with their associated unfed fry and smolt releases and the returning jacks and adults in return years 2003 to 2006. H×H and W×W represent the crosses performed in the hatchery in each of the three brood years, and W represents the wild fish sampled and passed above Nonpareil Dam in BY 2002 and 2003 that were allowed to reproduce naturally.



*OTS*3, *ONE*µ2, *OCL*8, *OTS*215, *ONE*µ13, *OMY*1011) following the methods of Moyer et al. 2007.

Parentage analysis, which was done separately for each brood year (BY; refers to the year in which the brood stock was sampled), was performed using a five-step approach. First, all returning offspring (ages 2 and 3) from a given BY were allocated to hatchery parental pairs using the software PAPA 2.0 (Duchesne et al. 2002). Allocations were performed based on maximum likelihood and were restricted by using the known structured mating design and a 2% error model distributed on the next adjacent allele. This error model takes into account scoring errors that usually occur by scoring the allele next to the true one. To take null alleles into account, we chose to add any allocation that yielded the most likely pair from the known hatchery mating design but was rejected by PAPA because of mismatches resulting from homozygous loci. Errors were more prevalent among *One*µ2 data, and frequency analysis showed evidence of null alleles for this locus with a frequency of 0.039 (MICRO-CHECKER; Van Oosterhout et al. 2004). Second, parentage analysis of any unassigned offspring from the previous step was performed using wild parents passed above Nonpareil Dam. Parentage analysis for this step was conducted using PASOS 1.0 (Duchesne et al. 2005). Analyses were only performed for BY 2002 and 2003 because Calapooya Creek was not sampled for wild coho salmon in BY 2001. Like PAPA, PASOS uses a maximum likelihood approach and an error model, but it differs from PAPA in that it also combines an exclusion approach and allocates parental pairs in an open system where some parents are potentially missing. We used a 2% error model and a similar procedure as described above for rejected parent pairs resulting from homozygote mismatches. Third, simulations were conducted using PASOS to estimate the number of missing wild spawners in 2002 and 2003 (see Duchesne et al. (2005) for description of simulation and Thériault et al. (2007) for an example). Fourth, to verify that the unassigned individuals from step 2 were of wild parents, we simulated wild offspring using the number of missing spawners estimated in step 3 for each year and allocated these offspring to hatchery pairs as detailed in step 1. The simulated offspring should not assign (or assign in very low proportion) to hatchery pairs if our allocation procedure is correct. Finally, the accuracy of our allocations was assessed via simulations as implemented in PAPA and PASOS as follows. Artificial offspring were produced from sampled parents used in this study and from simulated parents based on the number of missing spawners for each BY (for PASOS simulations only, because there are no missing hatchery spawners for PAPA simulations). Simulated offspring were then allocated to known sampled parents and the percentage of correctly assigned individuals was assessed.

Estimation and comparisons of survival rate

The number of offspring returning as 2- and 3-year-old adults divided by the total number released (i.e., survival rate) was compared between hatchery releases of unfed fry and smolts for each BY separately using a Yates corrected χ^2 test. We also compared survival rates among BYs to assess temporal differences. The number of returning offspring was obtained using the parental allocation described above.

Estimation and comparison of individual reproductive success

The average number of returning adult offspring produced per individual (i.e., individual reproductive success) was estimated from parentage analysis. Mean reproductive success of hatchery-spawned fish (number of returning offspring that were released as either unfed fry and smolts) was compared with mean reproductive success of fish spawned in the wild for BYs 2002 and 2003 (wild parents were not available in 2001). Estimates were calculated for both males and females combined (similar results were seen when data were analyzed by sex; data not shown). Significance in the difference between reproductive success among hatchery and wild fish and among BYs was assessed using two-tailed permutation tests as implemented in PERM 1.0 (Duchesne et al. 2006). Numbers of offspring were permuted 10 000 times, and the probability of obtaining a smaller or larger difference from the observed value was evaluated.

Comparison of life history traits

Size at age

Within each return year (RYs 2003–2005), average length (fork length) of returning fish to Calapooya Creek was compared for age-2 and age-3 fish, separately, among three groups as follows: returns stocked as fry, returns stocked as smolts, and wild returns (naturally occurring wild-born fish). For age-2 males (jacks), a one-way analysis of variance (ANOVA) was used to test for the effect of stocking history (fry, smolt, or wild) on length. If stocking was significant, we performed pairwise comparisons using a Student's *t* test. For age-3 returns, we first used a two-way ANOVA to test for the effect of stocking history and sex, as well as the interaction between these factors, on length. If the interaction was significant, a one-way ANOVA, which tested the effect of stocking history on length, was performed for each sex separately. If stocking was significant, then we performed pairwise comparisons using a Student's *t* test.

Run time

Similar to the length analysis, the date that progeny returned to Calapooya Creek (i.e., run time) was compared within each return year (RYs 2003–2005). Separate comparisons were made for age-2 and age-3 fish among the following three groups: returns stocked as fry, returns stocked as smolts, and wild returns. Run time was scored as the number of days after 1 October for each RY. For age-2 males, we used a Kruskal–Wallis test (run time is not normally distributed) on ranks for the effect of stocking history on run time. If stocking was significant, we used a nonparametric Wilcoxon test to compare each group. For age-3 fish, we first used a two-way ANOVA to test the effect of stocking history and sex, as well as the interaction between these factors, on run time. If the interaction term was significant, we performed a Kruskal–Wallis test on ranks for each sex separately. Furthermore, if stocking was significant, we tested each pairwise group comparison using a nonparametric Wilcoxon test.

Jacking

We also explored the incidence of returning age-2 males by comparing the proportion of jacks with that of the total number of male returns from each BY. Comparisons were made between each of three groups as follows: returns from unfed fry releases, returns from smolt releases, and wild returning individuals. Pearson's χ^2 analysis was used to assess significance. If significance was found, post hoc exact tests (Fisher) were performed to assess which group(s) was significant.

Results

Offspring from H×H and W×W crosses were pooled together and considered as F_1 hatchery fish, regardless of the origin of their parents, for all the results presented below. An ANOVA including parental captive history (H×H or W×W) and stocking strategy (unfed fry, smolt, or wild) revealed a significant interaction between these two effects in BY 2001 only (data not shown). However, there are no qualitative differences in any of our analysis when we separate by parental history, i.e., the patterns are the same and our conclusions are not affected whether the data were pooled or not (data not shown). Moreover, the low number of unfed fry jack returns necessitated the pooling of the data to provide sufficient sample size to fulfill requirements for statistical analysis. Captive parental history has already been dealt with in part in Moyer et al. (2007) with a subset of the data presented here.

Parentage analysis

A total of 1340, 1652, and 1639 progeny returned from BYs 2001, 2002, and 2003, respectively (Table 2). Twenty fish were excluded from analyses because they were missing tissue or genotypes for more than five loci. PAPA and PASOS allocation rates combined (i.e., percentage of fish that were allocated to parental pairs or single parent) were 34%, 87%, and 81% for BYs 2001, 2002, and 2003, respectively (the BY 2001 allocation rate was less than the other BYs because wild parents were unattainable). Simulations indicated that our allocation method was accurate because no wild offspring were falsely allocated to hatchery pairs using PAPA during the first step of our allocation process. The correctness rate (i.e., the percentage of fish allocated to the correct parents according to simulations) for allocation to hatchery parents in all three brood years using PAPA was 100%. The correctness rate for the allocation to wild parents using PASOS was 97% and 91% for BYs 2002 and 2003, respectively.

Approximately 3% of the marked returns were unassigned to a hatchery pair according to the known mating matrix (Table 2). The exact reason for these unresolved assignments is unclear, but it could be due to the spilling of gametes during hatchery spawning, mutations in offspring, genotype error, error in data collection or processing, or wrong age determination. Similarly, another 4% of the marked returns were dropped from analyses because of a mismatch between the mark and the hatchery pair assigned (for example, a fish marked adipose left maxillary should be assigned to a H×H pair but was assigned to a W×W pair). Because we did not want to prioritize the mark over the allocation results, or vice versa, we removed these fish from our analyses. Moreover, 38 returns from BY 2003 were, in fact, progeny of 66 F_1 jacks (returns from BY 2001) spawning in the wild in 2003. These fish were removed from subsequent analyses as they are F_2 returns.

Estimation and comparisons of survival rate

The survival rate for fish released as smolts was significantly greater than that of fish released as fry (X^2_{Yates} = 4351.7, 1161.79, and 6461.01 for BYs 2001, 2002, and 2003 respectively; $\chi^2_{0.05[1]} = 3.841$; all $p < 0.0001$; Table 1). Survival rates for unfed fry were also significantly different among BYs, where the survival rate for fry from BY 2003 was the highest, followed by BYs 2002 and 2001 (X^2_{Yates} = 26.87 for BY 2001 vs. BY 2002, 158.99 for BY 2001 vs. BY 2003, and 80.13 for BY 2002 vs. BY 2003; $\chi^2_{0.05[1]}$ = 3.841, all $p < 0.0001$); Table 1). Survival rate comparisons for smolts among BYs indicated that BY 2002 had the greatest survival followed by BYs 2003 and 2001 (X^2_{Yates} = 145.13 for BY 2001 vs. BY 2002, 70.81 for BY 2001 vs. BY 2003, and 14.8 for BY 2002 vs. BY 2003; $\chi^2_{0.05[1]}$ = 3.841, all $p < 0.001$; Table 1).

Estimation and comparison of individual reproductive success

The average individual reproductive success of brood stock whose offspring were released as unfed fry was significantly ($p < 0.001$) greater than that of fish that spawned in the wild for BY 2003 (Table 3). In contrast, average individual reproductive success for brood stock in BY 2002 was significantly ($p = 0.001$) less than that of the wild population in 2002. Average individual reproductive success of brood stock whose offspring were released as smolts was significantly ($p < 0.0001$) greater than that of fish that spawned in the wild for both BYs (Table 3). Comparisons across BYs

Table 2. Count of coho (*Oncorhynchus kisutch*) that passed above Nonpareil Dam (Calapooya Creek) for each brood year (BY).

BY	Total returns	Jacks returns (RY)	Adults returns (RY)	Assigned to H pairs	Assigned to W parents	Not used	Unresolved	Dropped
2001	1340	160 (2003)	1180 (2004)	439	866[*]	9	11	15
2002	1652	131 (2004)	1521 (2005)	900	686	7	27	32
2003	1639	165 (2005)	1474 (2006)	1020	580[†]	4	18	17

Note: The actual year of return (return year, RY) is given in parenthesis. The category labeled "numbers not used" corresponds to missing genotype information. Likewise, those labeled "unresolved" are marked fish unassigned, and those of the category "dropped" are marked fish that were misassigned. W, wild; H, hatchery.

[*] Identity of W spawners is not known. All fish not assigned to hatchery pairs are considered of W origin.

[†] Thirty-eight of the fish allocated to W parents were coming from 61 F_1 jacks (returns from BY 2001) that spawned in the wild in 2003. These 38 fish were removed from subsequent analysis, as they are, in fact, F_2 returns.

Table 3. Average individual reproductive success (RS) and relative reproductive success (RRS) of hatchery and wild coho salmon (*Oncorhynchus kisutch*) for brood years (BYs) 2002 and 2003.

	RS hatchery (SE)			RS wild (SE)		RRS	
BY	*N*	Fry	Smolt	*N*	Wild	Fry/wild	Smolt/wild
2002	400	1.04 (0.08)	3.46 (0.18)	783	1.41 (0.08)	0.74**	2.45**
2003	404	1.97 (0.14)	3.08 (0.15)	520	1.27 (0.1)	1.47**	2.43**

Note: RS and RSS were evaluated for unfed fry and smolt stocking strategies. **, RSS values significantly different from one at $p \leq 0.001$.

Table 4. Mean fork length (mm) of coho (*Oncorhynchus kisutch*) that passed above Nonpareil Dam for each brood year (BY), according to their stocking origin (fry, smolts, or wild born).

	Fry		Smolt		Wild	
BY	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)
Jacks (age 2)						
2001	5	406.0 (24.8)	61	420.6 (27.0)	94	411.2 (38.0)
2002	7	425.7 (22.4)	66	421.2 (33.2)	56	417.8 (55.8)
2003	20	443.3 (30.9)	67	428.2 (28.7)	72	445.3 (28.8)
Adults (age 3)						
2001	74	728.6 (62.3)	299	711.6 (61.1)	784	723.2 (55.8)
2002	200	709.9 (66.2)	627	700.3 (63.2)	657	716.9 (60.7)
2003	377	739.5 (57.3)	555	719.3 (56.9)	488	743.2 (60.7)

Table 5. Two-way analysis of variance (ANOVA) results for age-3 coho comparing length between stocking origin (fry, smolts, or wild born) and sex for each brood year (BY).

Source	df	Sum of squares	*F* ratio	*p*
BY 2001				
Stocking	2	32 692.43	4.92	0.007
Sex	1	3 414.54	1.03	0.311
Stocking × sex	2	3 583.30	0.54	0.583
BY 2002				
Stocking	2	93 636.27	12.30	<0.0001
Sex	1	115 369.26	30.30	<0.0001
Stocking × sex	2	21 957.14	2.88	0.056
BY 2003				
Stocking	2	180 206.56	26.96	<0.0001
Sex	1	15 957.76	4.77	0.029
Stocking × sex	2	16 299.35	2.44	0.088

for average individual reproductive success of brood stock whose offspring were released as unfed fry showed that BY 2003 was significantly greater than BY 2002 ($p < 0.0001$). The average individual reproductive success estimate for brood stock whose offspring were released as smolts was significantly ($p = 0.02$) greater in BY 2002 than in BY 2003. There was no difference ($p = 0.18$) in the estimate of average individual reproductive success for wild fish between the two years sampled.

Comparison of life history traits

Size at age

The average length for fish passed above Nonpareil Dam each year, according to their stocking origin, are shown (Table 4). There was a significant difference (all $p < 0.01$) in average length for jacks stocked as fry, smolts, or naturally occurring in the wild for BY 2003. The significant difference in length for progeny of BY 2003 can be explained by an increase in average length of jacks returning from fry releases and from naturally spawned individuals when com-

Table 6. Mean run time (number of days since 1 October of each run year) of coho that passed above Nonpareil Dam for each brood year (BY), according to their stocking origin (fry, smolts, or wild born).

	Fry		Smolt		Wild	
BY	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)
Jacks (age 2)						
2001	5	64.2 (7.4)	61	61.7 (6.4)	94	67.1 (10.4)
2002	7	61.3 (16.4)	66	51.7 (18.1)	56	66.6 (14.8)
2003	20	57.0 (13.8)	67	53.0 (15.2)	72	59.9 (15.2)
Adults (age 3)						
2001	74	65.8 (13.6)	299	58.1 (18.6)	784	71.1 (12.0)
2002	200	54.3 (13.6)	627	52.7 (12.8)	657	63.3 (15.4)
2003	377	45.7 (8.7)	555	44.5 (7.3)	488	53.0 (18.9)

Table 7. Two-way analysis of variance (ANOVA) results for age-3 coho comparing run time between stocking origin (fry, smolts, or wild born) and sex for each brood year (BY).

Source	df	Sum of squares	*F* ratio	*p*
BY 2001				
Stocking	2	36 449.04	92.04	<0.0001
Sex	1	520.22	2.63	0.1053
Stocking × sex	2	2.96	0.008	0.9926
BY 2002				
Stocking	2	38 134.54	96.35	<0.0001
Sex	1	796.75	4.03	0.045
Stocking × sex	2	76.43	0.19	0.824
BY 2003				
Stocking	2	19 914.75	125.82	<0.0001
Sex	1	2 553.12	32.26	<0.0001
Stocking × sex	2	204.17	1.29	0.2756

pared with that of jacks returning from smolt releases (smolts vs. wild, $p = 0.0007$; smolts vs. fry, $p = 0.044$). Note that for BYs 2001 and 2002, sample sizes were very small for fry ($n = 5$ and 7, respectively), limiting our power to determine significant size differences.

There was a significant difference (all $p < 0.01$) in length for age-3 returns stocked as fry, smolts, or naturally produced in the wild for all BYs (Table 5; there was no significant interaction for stocking origin and sex of fish). For all BYs, significant differences in length at return is attributed to an increase in average length of adults returning from fry releases and from naturally spawned individuals when compared with that of adults returning from smolt releases (BY 2001: smolts vs. wild, $p = 0.003$; smolts vs. fry, $p = 0.021$; BY 2002: smolts vs. wild, $p < 0.0001$; smolts vs. fry, $p = 0.06$; BY 2003: both comparisons, $p < 0.0001$).

Run timing

Mean run time of fish passed above Nonpareil Dam each year according to their stocking origin are shown (Table 6). There were significant differences (all $p < 0.01$) in run time for jacks stocked as fry, smolts, or naturally occurring for BYs 2001, 2002, and 2003. Differences in run time were attributed to a significantly (all $p < 0.01$) earlier average run time in progeny released as smolts when compared with that of progeny from naturally spawning coho salmon. Again, small sample sizes for fry in BYs 2001 and 2002 limit our analytical power when considering pairwise comparisons with fry.

There were significant differences (all $p < 0.0001$) in run time for age-3 returns stocked as fry, smolts, or naturally occurring in the wild for all BYs (Table 7; there was no significant interaction for stocking origin and sex of fish). Significance for BY 2001 is attributed to a different run time for every group of fish. On average, adults stocked as smolts returned to Nonpareil Dam earliest, followed by adults stocked as fry and then naturally occurring adults (all $p < 0.001$; Fig. 3). For BYs 2002 and 2003, differences were attributed to a later average run time of naturally occurring adults (all $p < 0.0001$).

Jacking

We found significant (all $p < 0.01$) differences in the proportion of jacks produced from progeny stocked as fry versus progeny stocked as smolts for each BY (Fig. 4). Post hoc testing revealed that the probability of being a jack was greater for an individual released as a smolt than for an individual released as a fry (all $p < 0.01$). When comparing the incidence of jacking between progeny stocked as fry and those naturally occurring in the wild, significant (all $p < 0.01$) differences were found in BYs 2002 and 2003 (Fig. 4). For both BYs, post hoc testing revealed that the probability of being a jack was greater for wild offspring than for hatchery offspring released as fry (all $p < 0.01$). The incidence of jacking was also different between individuals stocked as smolts and those naturally occurring in the wild for BY 2001 ($p = 0.008$). Individuals stocked as smolts had a greater probability of being a jack than did naturally occurring fish ($p = 0.006$).

Discussion

Evaluation of hatchery strategies

The main goal of this study was to evaluate the success of an integrated conservation hatchery program (i.e., random 1:1 paired matings and equalized parent contributions) that implemented two different stocking strategies — outplanting unfed fry and smolts. One of the primary measures of success for such a program is the evaluation of reproductive success between stocking strategies and between hatchery

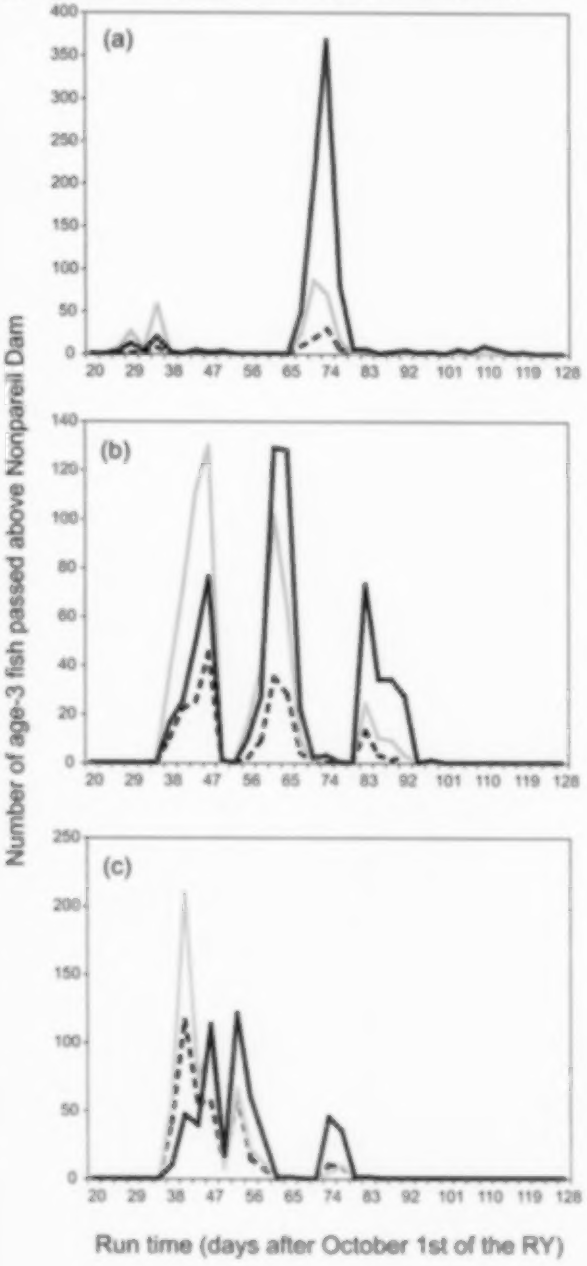


Fig. 3. Run time of age-3 coho (*Oncorhynchus kisutch*) returning to Calapooya Creek for (*a*) brood year (BY) 2001, returning in run year (RY) 2004; (*b*) BY 2002, returning in RY 2005, and (*c*) BY 2003, returning in RY 2006 according to their stocking origin (fry, broken line; smolts, shaded line; wild-born, continuous line).

and wild components. A successful hatchery program should increase egg-to-adult survival over naturally produced fish because the hatchery is intended to minimize mortality at critical early life history stages, otherwise observed in the natural population. We found this to be the case for smolt releases and one of two unfed fry releases.

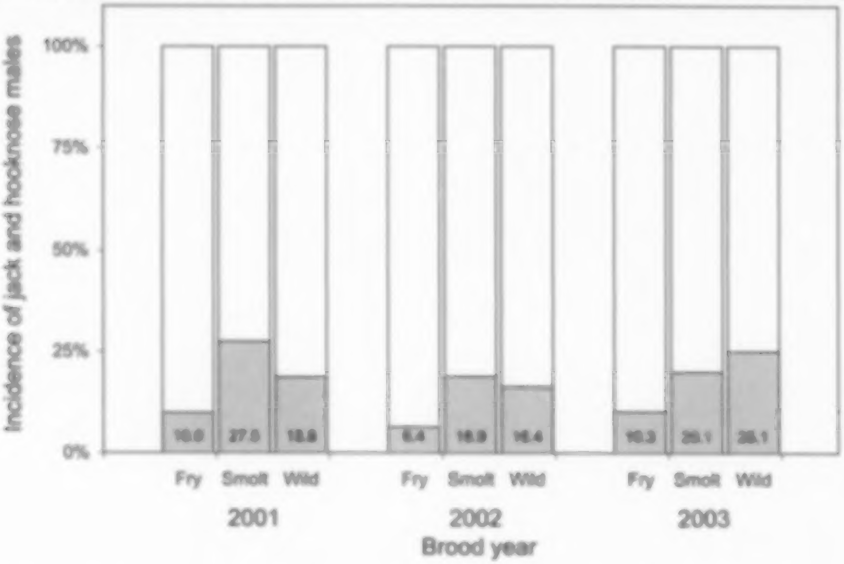
A significantly lower estimate of RRS for hatchery fish whose progeny were stocked as fry in BY 2002 could be explained by detrimental environmental conditions experienced by hatchery fry once supplemented in the river. Indeed, time of release in 2003 (for BY 2002) corresponded to a period of higher flows than for releases in 2004 (for BY 2003) (L. Jackson, ODFW, Roseburg, Oregon, USA, personal communication). Data from smolt releases in 2003 also support this hypothesis because they had the lowest survival rate of the three years under study. The fact that only hatchery fry and not wild fry would have been impacted by these higher flows could result from the prior residence of wild fry in safer territories or because wild fry may still have been in the gravel. Regardless, our data showed that the unfed fry release strategy appeared to increase the number of returns relative to wild spawning under certain conditions (BY 2003); however, the increase was not as substantial as with stocking smolts.

A caveat to our findings is that the estimate of RRS could be influenced by the relative number of offspring released as smolts versus unfed fry per family group. For example, in our study, approximately 95% of the gametes produced by an individual were released as unfed fry. If more smolts were released per paired mating, then there exists a possibility that the expected RS could be greater than our observed value, although the conclusion would be in the same direction (i.e., the smolt strategy increases RS relative to wild spawning). Unfortunately we have no way of estimating RS on a per-gamete basis (e.g., if 100% of the offspring from a paired mating were released as smolts) because survival is likely density-dependant. Therefore we urge caution when applying our RRS results to other such hatchery programs, especially those releasing a large portion of smolts relative to fry, unless density-dependent factors influencing survival are known.

Our study also found important differences in survival depending on the hatchery release strategy. On average, returning fish released as smolts had a significantly greater survival rate (25- to 75-fold) compared with fish released as unfed fry. This finding is consistent with our expectations because hatcheries keep juvenile coho salmon in captivity for longer periods to increase survival during the critical freshwater life stage that unfed fry would experience outside the hatchery (Unwin 1997). It is important to note that we compared survival of unfed fry to adulthood with survival of smolts to adulthood. Acknowledging that this is an unbalanced comparison, we are unable to discern if survival from smolt to adulthood is different between fish stocked as unfed fry and those stocked as smolts. Therefore, we were unable to offer direct support for the 10-fold increase in survival to adulthood described in Rideout and Stolte (1988), where smolts from fry releases were shown to be better adapted to facing oceanic migration than fish released as smolts.

Increased survival and RRS are not the only parameters that should define success of a conservation hatchery program. Maintaining genetic diversity from generation to generation is also important. For example, if the majority of smolts that survived to adulthood were from a few paired matings, a considerable loss of genetic diversity would be

Fig. 4. Incidence of jacks (age 2) compared with hooknose (age 3) among males that returned to Calapooya Creek for each brood year that were stocked as fry, smolts, or wild born. The numbers within the bars represent the percentage of jacks for each group.



associated with the program. Although this study does not deal with the conservation of genetic diversity (see Moyer et al. 2007), it provides insight into the efficiency of the unfed fry release strategy, which has not been rigorously evaluated, despite decades of STEP implementation in the Pacific Northwest. From a conservation genetics viewpoint, we note that alternate rearing strategies may have different outcomes depending on a myriad of genetic and environmental parameters and interactions. We illuminate this consideration by comparing smolt and fry releases with wild fish for three different life history characteristics.

Evaluation of life history characteristics

In this study, wild-born fish and fish stocked as unfed fry had the same average length at return, whereas smolts were significantly smaller. Smaller size at return for hatchery smolts has been reported in other hatchery programs (McLean et al. 2004; Knudsen et al. 2006). There are several competing hypotheses (none of which is mutually exclusive) that explain these differences. First, hatchery smolts could experience depressed growth during their transition from artificial to natural food. In turn, this depressed growth may not be recovered later in life. Second, hatchery smolts could have a slower outmigration rate than their wild counterparts that would translate into earlier and increased feeding opportunities and subsequent greater growth of wild fish in the ocean phase of their life cycle. Third, hatchery smolts could also experience an earlier run time than wild fish, causing hatchery smolts to spend less time at sea. Finally, there could be a genetic component if length was a heritable trait such that small brood stock (when compared with the wild) on average produced smaller offspring.

Though it is difficult to disentangle competing hypotheses that might explain our length data, we can eliminate the latter hypothesis because if there were a genetic effect due to the heritability of length, then we would expect that the average length of the hatchery brood would be significantly less than that of the wild population. Instead, we saw that fish used as brood stock for our study were significantly larger than the wild fish (data not shown), indicating that there is a strong environmental influence on length of returning hatchery fish.

We also found significant differences in run timing among returning progeny from smolts when compared with unfed fry releases and the wild population. Although run time overlapped for all groups, fish stocked as smolts returned earlier to their stream of origin, followed closely by fish stocked as fry, and then by wild fish. Earlier run timing of hatchery fish has been observed in other hatchery programs (Unwin and Glova 1997; McLean et al. 2003; Ford et al. 2006). Run timing is a heritable trait in salmonids with the capacity to rapidly evolve and adapt to local environmental conditions (Hansen and Jonsson 1991; Quinn et al. 2000; Carlson and Seamons 2008). Relaxation of natural selection on run timing in the hatchery environment may potentially lead to the survival of certain genotypes and phenotypes that would otherwise be selected against in nature. For example, early spawning in nature can be selected against because of redd disturbance by later spawners (van den Berghe and Gross 1989) or because fry that emerge too early may face limiting food resources or unsuitable stream flow conditions (Nickelson et al. 1986). These selection processes do not take place in the hatchery environment. Additionally, artificial selection resulting from hatchery managers that unintentionally spawn the earliest fish caught as brood stock to secure a brood stock quota could result in an over-representation of early run-time phenotypes.

Differences in run time found in our study appeared to be the result of direct artificial selection. Despite efforts to collect a representative sample from the entire spawning run, brood stock used in this study were captured at a significantly earlier date. Brood stock sampled at Winchester Dam and Nonpareil Dam had a run time on average 46 and 6 days earlier, respectively, than the natural run in Calapooya (data not shown). Differences between hatchery and wild fish are likely the result of passing these differences from parent to offspring. Therefore, we would expect that fry releases would show the same pattern as smolts, as they have the same genetic background. Although returns from fry releases were consistently later than returns from smolt releases, generally, the trend was not significantly different. Discrepancies could be artifacts of low sample size (fry returns) or because environmental influences on run time were greater for fry releases.

Differences in the incidence of jacking between stocking strategies may be attributed to complex interactions among environmental and genetic effects. Alternative mating tactics in salmonids often associated with early maturation have been shown to be heritable (Silverstein and Hershberger 1992; Heath et al. 1994; Wild et al. 1994) but are also influenced by environmental conditions acting on growth and other energetic traits (Rowe and Thorpe 1990; Forseth et al. 1999; Olsson et al. 2006). It is well established that age at maturity is negatively correlated with growth rate in fishes (Stearns and Koella 1986; Hutchings 2004), and fast growth has been shown to be associated with a greater probability of jacking (Vøllestad et al. 2004). Based on these findings, we would expect fish reared to the smolt stage to exhibit the highest incidence of jacking because of their faster growth in the hatchery environment (for example, see Unwin and Glova 1997; Larsen et al. 2004; Knudsen et al. 2006). Our data supported these conclusions. The incidence of jacking was greater in fish stocked as smolts than in those stocked as fry. We also noted a higher tendency for fish stocked as smolts to return as jacks in BYs 2001 and 2002 than for wild fish, but this tendency is reversed in BY 2003. In 2003, 44% of the wild males passed above Nonpareil Dam were jacks. This was a much greater percentage than reported in any other year among available data (average of 17% over 3 years). However, only 16% of the males sampled for hatchery brood in 2003 were jacks. If we consider jacking to be heritable, then the greater incidence of jacking among wild returns from BY 2003 could be the result of an increased proportion of natural jack spawning among BY 2003 relative to the proportion spawned in the hatchery. Similarly, lower incidences of jacking from hatchery fry releases compared with the wild (when we would expect them to be similar because these two groups are hypothetically experiencing similar environmental conditions) may be the result of fewer jacks spawned among hatchery crosses relative to the natural population (BY 2002, 7% of the hatchery males were jacks, whereas 17% of the wild spawning males were jacks; see above for BY 2003; no data on wild fish were available for BY 2001).

Our findings illustrate that juvenile release strategies in supplementation programs have important consequences for coho salmon survival rates, numbers of adult returns, and other key life history characteristics. Irrespective of the genetic or environmental origin of these differences, any changes in these critical features are likely to affect the fitness of the population (Stearns 1992; Goodman 2005). Accumulating evidence demonstrates reduced fitness of hatchery fish in the wild (Araki et al. 2008; Araki et al. 2009), and researchers are currently exploring causal mechanisms. Overall, our study indicated that a fry stocking strategy produced fish more similar to the wild component of the population than that of fish released as smolts. However, the extent to which hatchery fish are influenced by the differences found in life history traits, once they reproduce in the wild, remains unresolved. Assessing the relative reproductive success in the wild of unfed fry releases versus smolt releases and wild-born fish is critical for a complete understanding of the efficiency of the two release strategies.

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Thermal reaction norms in sperm performance of Atlantic cod (*Gadus morhua*)

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Abstract: Phenotypic plasticity occurs when a genotype produces variable phenotypes under different environments; the shapes of such responses are known as norms of reaction. The genetic scale at which reaction norms can be determined is restricted by the experimental unit that can be exposed to variable environments. This has limited their description beyond the family level in higher organisms, thus hindering our understanding of differences in plasticity at the scale of the individual. Using a three-year common-garden experiment, we quantify reaction norms in sperm performance of individual genotypes within different families of Atlantic cod (*Gadus morhua*). Cod sperm showed phenotypic plasticity in swimming performance across temperatures (3, 6, 11, and 21 °C), but the pattern of the response depended upon how long sperm had been swimming (30, 60, 120, or 180 s), i.e., plasticity in plasticity. Sperm generally swam fastest at intermediate temperatures when first assessed at 30 s after activation. However, a significant genotype × environment interaction was present, indicating inter-individual differences in phenotypic plasticity. To our knowledge, this is the first study to describe variable sperm performance across environmental conditions as a reaction norm. The results have potential theoretical, conservation, and aquaculture implications.

Résumé : Il se produit une plasticité phénotypique lorsqu'un génotype détermine des phénotypes qui varient en fonction des différents environnements; les formes de telles réactions sont connues sous le nom de normes de réaction. L'échelle génétique à laquelle les normes de réaction peuvent être déterminées est restreinte par l'unité expérimentale qui peut être exposée à des environnements variables. Cela a limité leur description au-delà du niveau de la famille chez les organismes supérieurs, ce qui a entravé notre compréhension des différences de plasticité à l'échelle des individus. Dans une expérience de trois ans en jardin commun, nous avons mesuré les normes de réaction dans la performance des spermatozoïdes de génotypes individuels dans différentes familles de morues franches (*Gadus morhua*) de l'Atlantique. Les spermatozoïdes de morues font montre de plasticité phénotypique dans leur performance de nage sur une gamme de températures (3, 6, 11, et 21 °C), mais le patron de la réaction dépend du temps que le spermatozoïde a déjà consacré à la nage (30, 60, 120, ou 180 s), c'est donc une plasticité dans la plasticité. Les spermatozoïdes nagent généralement le plus vite aux températures intermédiaires lorsqu'ils sont évalués 30 s après leur activation. Cependant, il existe une interaction significative génotype × environnement, ce qui indique qu'il y a des différences de plasticité phénotypique entre les individus. À notre connaissance, notre étude est la première à décrire une performance variable des spermatozoïdes comme des normes de réaction sur une gamme de conditions du milieu. Nos résultats ont des incidences théoriques potentielles, mais aussi des conséquences sur la conservation et l'aquaculture.

[Traduit par la Rédaction]

Introduction

The capacity for individuals and populations to respond to spatial and temporal environmental fluctuation is paramount to species persistence. Although populations can evolve through time, individuals cannot undergo genetic change and their reproductive success can be severely jeopardized under suboptimal conditions. Individual fitness will decline with environmental change if the optimal expression of an important trait is rigid and requires specific conditions (Ghalambor et al. (2007) and references therein). Within this context, it has long been recognized that traits with flexible expression may enable long-term existence under unpredictable environments (Baldwin 1896; Morgan 1896). How large-scale environmental perturbations such as climate change affect species persistence will depend to some degree on the ability of individuals and populations to respond and adapt to such changes.

Phenotypic plasticity occurs when a given genotype produces variable phenotypes under different environments

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(Schlichting and Pigliucci 1998; West-Eberhard 2003). Phenotypes may represent continuous (e.g., colour intensity, growth rate) or discrete (e.g., colour morphs, alternative maturation strategies) traits, and plasticity can be adaptive, maladaptive, or nonadaptive (neutral), depending on the trait and context (reviewed by Ghalambor et al. 2007). Plasticity can be represented as a reaction norm (also known as a norm of reaction), which serves to quantify and visualize the linear or nonlinear shape of the phenotypic response to the environment (Woltereck 1909; Schlichting and Pigliucci 1998).

In addition to mean trait values, reaction norms themselves may be under selection (Scheiner 1993; Hutchings 2004), as the optimum phenotypic expression of a given trait may vary under unpredictable environments. Arguments have been made that reaction norms both hinder and promote adaptation (Price et al. 2003; Ghalambor et al. 2007). For example, if adaptive phenotypic plasticity shifts mean trait expression to optimal levels in each environment, the genotype is protected from selection and evolution does not occur. In contrast, adaptive plasticity may enable trait expression to move towards, but not reach, the optimum in a different environment. This allows individuals to persist, thus enabling directional selection to further pull the trait towards optimality. Additionally, nonadaptive plasticity can promote rapid evolutionary change (Ghalambor et al. 2007) if novel phenotypes are produced at extreme environments through the release of cryptic genetic variation (Queitsch et al. 2002; Rutherford 2000, 2003).

Reaction norms vary temporally and spatially within species, and although genetic differences have been examined in limited detail, they are likely ubiquitous. Examples include physiological traits such as growth rate in caterpillars (Kingsolver et al. 2006) or energy allocation in fish (Purchase and Brown 2001), morphological traits such as colour patterns in flies (Ottenheim et al. 1996), and behavioural traits such as reproductive signaling rates in moths (Jia et al. 2000). The resolution at which genetic differences in reaction norms persist is generally unknown but can occur at the population level on finer scales than revealed by selectively neutral DNA markers (Hutchings et al. 2007). Genetic differences in reaction norms among populations would indicate how individuals, on average, differentially respond to environmental variation. They can also vary among full- or half-sibling families, as indicated by studies on plants (e.g., Agrawal 2001; Waller et al. 2008), invertebrates (e.g., Kingsolver et al. 2006; Engqvist 2008), and a very small number of vertebrates (Beacham and Murray 1985; Yamahira et al. 2007).

We argue that sexually reproducing animals with external fertilization have sperm that are ideal for studying reaction norms. Our search of the literature (March 2009) revealed no published work on sperm quality framed in this context, but see Engqvist (2008) for a study on sperm quantity in scorpion flies. The evolutionary utility of sperm research has recently been summarized by Birkhead et al. (2009). We feel that there are three major advantages to studying reaction norms of sperm quality. First, for many species, males provide nothing to the next generation except sperm, and the genetic material within, to fertilize eggs. In such cases, fertilization ability is the ultimate expression of male quality. Sperm have measureable characteristics such as swimming velocity that are often tightly linked to fertilization success. Therefore, studies can be conducted on traits that not only directly relate to fitness, but also represent the compilation of a series of life processes that may have taken decades to achieve. Secondly, single cells are likely more sensitive to environmental variation than the whole organism, and thus reactions norms can be determined on finer scales. Unlike many other types of cells, it is relatively easy to assess the impact of the environment on sperm. Lastly, although by definition phenotypic plasticity is a function of the genotype, in reality it is not possible to compare genotypes in many cases. For most sexually reproducing animals, the family level is the finest genetic scale upon which reaction norms can be studied, and in such cases, environmental gradients are tested among siblings (i.e., among closely related genotypes). Although individual sperm from an ejaculate share only 50% of the haploid genetic material that they contribute to the next generation (Parker 1993; Parker and Begon 1993), sperm morphology (Pitnick et al. 2009) and swimming "behaviour" (Haig and Bergstrom 1995; Bernasconi et al. 2004; Immler 2008) are thought to be under diploid control of the father (also see Presgraves (2009) and references therein). Therefore, if different sperm from a single male are subjected to a variety of controlled environments, reaction norms can be genetically controlled in a way that is somewhat analogous to clonal organisms (see Scheiner 1993). Such studies are powerful as they are capable of testing environmental gradients within siblings (i.e., within genotypes or intra-individual), which fits within the theoretical construct of phenotypic plasticity.

We undertook a two-stage, three-year common-garden experiment to investigate reactions norms of sperm quality in a marine fish. Using three families of Atlantic cod (*Gadus morhua*), we addressed three hypotheses: (*i*) there is phenotypic plasticity in sperm performance with temperature, and a potential response to temperature depends on how long sperm have been swimming, (*ii*) there is genetic variability in the thermal reaction norm, and (*iii*) extreme environments promote novel phenotypes. Our results document reaction norms of individual genotypes and have potential theoretical, conservation, and aquaculture implications.

Materials and methods

Study organism

Atlantic cod are widely distributed on both sides of the North Atlantic Ocean, but recent evidence indicates that populations show much more homing to spawning sites than previously believed (Robichaud and Rose 2001; Svedäng et al. 2007). Males provide nothing but sperm to reproduction, and although cod are known to form dense spawning aggregations (if males lek, they may help choose spawning sites), details of spawning behaviour have not been documented in the wild. In captivity, research has shown that there is male display, male–male competition, female mate selection, and males that sneak fertilizations (Brawn 1961; Hutchings et al. 1999). Sperm competition is intense (Hutchings et al. 1999), and multiple paternity of a single batch of eggs does occur in tank spawnings (Rakitin et al. 1999*a*, 2001; Herlin et al. 2008). Under sperm competition, most egg fertilizations likely occur within seconds of gamete release in natural situations, and sperm swimming speed is therefore expected to be linked to fitness. However, why cod sperm can remain viable for an unusually long time (Trippel and Morgan 1994) is unknown.

General framework of experiment design

An efficient way of disentangling environmental influences from genetic influences on phenotypic variation is to use common-garden experiments. In such studies, individuals from different groups (e.g., populations, families) are kept under identical environmental conditions. Phenotypic differences among groups would therefore indicate a genetic and, if positively related to fitness, adaptive basis. If multiple environments are used, observed phenotypic differences in such experiments can result from three sources: the environment (E), the genotype (G), or their interaction (G × E). A significant G × E interaction would indicate differences among groups in how the phenotypic trait(s) responds to a range of environments and thus indicates genetic variation in phenotypic plasticity. Common-garden experiments are regularly undertaken with short-lived organisms such as annual plants but are rare in long-lived vertebrates because of practical complexities and expense.

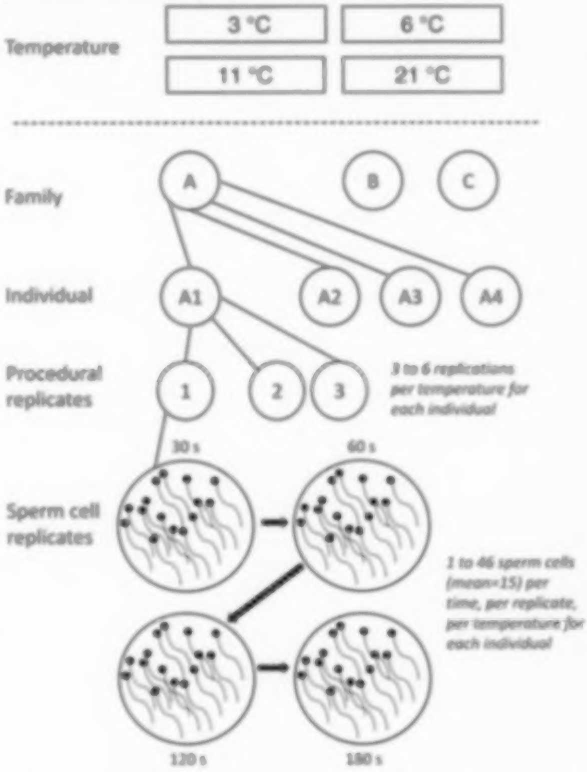
Common-garden phase I

Source of fish

Wild cod were captured from the Bay of Fundy (Canada) in 2004 and brought to the Fisheries and Oceans Canada Biological Station in St. Andrews, New Brunswick (45°N, 67°W). These fish were kept under ambient temperature and lighting conditions and were fed a mixture of squid and mackerel. In February and March of 2005, gametes were stripped from these fish and different full-sib families were created from in vitro fertilizations. Standard aquaculture protocols were used for embryo incubation (~6 °C) and larval rearing (~10 °C). Each family was initially kept in a different incubator and larval rearing tank but was subjected to the same conditions in a common room. At about 6 months in age, each juvenile was implanted with a passive integrated transponder (PIT) tag and placed in a common tank (families combined) in September of 2005. These fish were raised in a flow-through seawater system under ambient photoperiod and salinity and fed marine grower pelletized diet (EWOS Canada, Surrey, British Columbia). Temperature was maintained between 2 and 4 °C leading up to and during the spawning season in 2008.

Each individually tagged cod was assessed on 9 January 2008 to determine total body length (±0.1 cm) and weight (±1 g) prior to commencement of the spawning season at three years of age. The sex of each fish and its maturation status were determined using a small sample of gametes obtained by cannulation. Four brothers (analogous to individual genotypes and hereafter referred to as individuals) were chosen from each of three families for the experiment (Fig. 1). Specific individuals were selected to minimize size differences among families (Table 1), as the swimming ability of cod sperm may be influenced by some measures of fish "size" (see Discussion for references).

Fig. 1. Design of the phase II common-garden experiment. Shown is the hierarchical structure for a single temperature. There were three families of cod, each containing four individuals. Sperm were obtained from one brother from each family at the same time on a given day. The sperm activation procedure was repeated at least three times for each fish, and the movement of different individual sperm was determined at four time periods per activation. This entire process was repeated for the four manipulated temperatures each day, the testing order of which was randomized among days (different brothers used on different days).


Temperature
3 °C
6 °C
11 °C
21 °C
Family
A
B
C
Individual
A1
A2
A3
A4
Procedural replicates
1
2
3
3 to 6 replications per temperature for each individual
30 s
60 s
Sperm cell replicates
1 to 46 sperm cells (mean=15) per time, per replicate, per temperature for each individual
120 s
180 s

Collection of semen for sperm experiments

The assessment of sperm was conducted between 25 February and 8 March 2008. One individual was assessed for each family per day (three fish per day, four experimental days). The target fish were anesthetized with MS-222 (tricaine methanesulfonate) to minimize stress during handling and stripping. The external urogenital pore was wiped dry, and semen was obtained by applying slight pressure on the abdomen. To help avoid seawater, urine, and feces contamination, the first 1–2 mL of ejaculate was discarded in a standardized manner. Approximately 10 mL of ejaculate was then collected in a 50 mL sterilized dry Pyrex beaker and immediately covered with aluminum foil and temporarily placed in a cooler at 6 °C. Samples were then held in a 5–7 °C environmental chamber until sperm assessment.

Spermatocrit (defined as the ratio of packed white cells to the total volume of semen × 100) was used to estimate spermatozoa density (Rakitin et al. 1999*b*). Semen from each male was drawn into three microhaematocrit capillary tubes (75 mm length, 1.1–1.2 mm internal diameter). One end of

Table 1. Characteristics of each individual genotype (i.e., brother) from full-sib families of Atlantic cod (*Gadus morhua*) used in the sperm analyses and mean ± standard deviation (SD) for each family.

Individual	Total length (cm)	Body weight (g)	Fulton's condition[b]	Spermatocrit (%)	Mean VCL ($\mu m \cdot s^{-1}$)[c]
Family A[a]					
A1	51.7	1575	1.14	34.3	59.1
A2	46.4	1230	1.23	34.0	58.2
A3	45.8	1075	1.12	26.3	65.0
A4	42.3	920	1.22	41.0	49.7
Mean ± SD	46.6 ± 3.9	1200 ± 280	1.18 ± 0.06	33.9 ± 6.0	58.0 ± 6.3
Family B					
B1	47.0	1335	1.29	39.7	42.0
B2	48.7	1365	1.18	32.7	48.6
B3	51.7	1830	1.32	37.0	55.3
B4	48.2	1275	1.14	59.0	47.6
Mean ± SD	48.9 ± 2.0	1451 ± 255	1.23 ± 0.09	42.1 ± 11.6	48.2 ± 5.7
Family C					
C1	47.1	1260	1.21	40.7	38.2
C2	45.5	1100	1.17	35.0	54.2
C3	44.7	1020	1.14	11.3	55.7
C4	46.6	1445	1.43	49.3	43.7
Mean ± SD	46.0 ± 1.1	1206 ± 188	1.24 ± 0.13	34.1 ± 16.3	48.0 ± 8.5

[a] Families were not significantly different in any of the general parameters (one-way analyses of variance (ANOVAs): $P_{\text{length}} = 0.290$, $P_{\text{weight}} = 0.301$, $P_{\text{condition}} = 0.635$, $P_{\text{spermatocrit}} = 0.568$); see Table 2 for statistics on VCL.

[b] Fulton's condition factor is equal to $100(\text{weight} \cdot \text{length}^{-3})$ and represents relative weight for a given length.

[c] Mean VCL is sperm curvilinear swimming velocity averaged across replicates, temperatures, and times.

each tube was sealed with Critoseal (Krackeler Scientific, Inc., Albany, New York), and the tubes were centrifuged for 10 min at 7500 rpm. The mean of the three measurements per male created a single datum (Table 1) that was used for statistical analysis.

Common-garden phase II

Sperm exposure to temperature

Four test temperatures were used (3 ± 1, 6 ± 1, 11 ± 1, 21 ± 1 °C) in sperm assessment (Fig. 1), which were chosen based on a compromise between a wide comparison range and the limits of our facility. Cod will naturally spawn at 3 °C and 6 °C but do not reproduce at 21 °C as they never experience this temperature in the wild. The experiment was conducted in a series of four temperature-controlled rooms. The microscope, activating medium, slides, cover slips, and pipette tips were acclimated to room temperature before sperm activation. Semen from each male was transferred from the storage beaker (~6 °C) into plastic tubes and placed in the test room for 15–20 min before activity trials commenced (preliminary experiments showed that semen slowly reached test temperature over this time period). Each semen sample was tested at the four temperatures (repeated measures). The test order of the temperatures was randomized among the four days (one individual from a given family on each day): day 1 test order was 21, 11, 3, and 6 °C; day 2, 3, 11, 21, and 6 °C; day 3, 11, 6, 21, and 3 °C; and day 4, 6, 3, 11, and 21 °C. Temperature of the swimming sperm was continually and precisely monitored during the experiment using an infrared temperature gun aimed at the edge of the slide cover slip.

All sperm activity tests were completed within 12 h of semen collection. Rouxel et al. (2008) reported that a significant decrease in Atlantic cod sperm motility does not occur until after 168 h of storage at 4 °C. Therefore, we feel confident that the maximum of 12 h delay in sperm activation after collection had a minimal affect on sperm quality in our experiment. Moreover, the study was designed such that any effect would not bias comparisons among temperatures or families but would appear as variability among individuals within a family (see Discussion).

Sperm activation procedure

Fish sperm is inactive in vivo and remains inactive until in contact with water of appropriate chemistry (Cosson et al. 2008). The activating medium was composed of 32 ppt seawater plus 1% *w/v* bovine serum albumin (BSA). BSA was added to prevent sperm from sticking to the glass slides (Rouxel et al. 2008). Air was bubbled into the activating medium to maintain dissolved oxygen levels at saturation. Repeated measurements indicated that the pH ranged from 7.18 to 7.20.

Sperm activity was induced by pipetting 0.5 µL of semen into a plastic tube containing 300 µL of activating medium. The semen and activating medium were shaken for 5 s to homogenize the sample. We pipetted 40 µL of this dilution into a well of a 10-well multitest glass slide (MP Biomedicals, Solon, Ohio) and put a cover slip in place.

Sperm from many fish species are active for very short periods of time. When there is sperm competition, as in cod, swimming speed should influence fertilization ability more than swimming longevity (Rudolfsen et al. 2006), and most fertilizations should occur within seconds. In our ex-

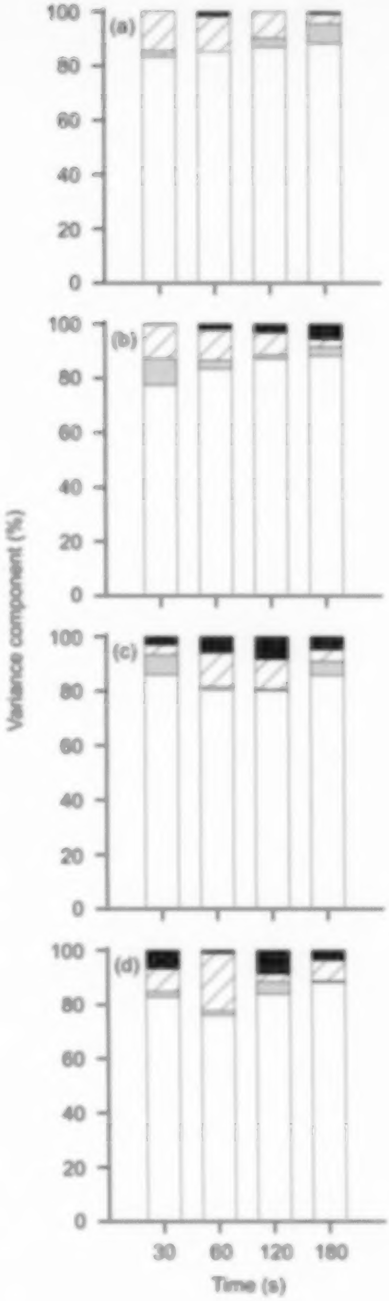


Fig. 2. Results of 16 fully nested analyses of variance (ANOVAs) separating variance components for sperm swimming velocity (VCL) among families (solid bars), individuals within families (hatched bars), procedural replicates within individuals (shaded bars), and sperm cells within replicates (open bars). Four time periods are given on the *x* axis; each assessment was conducted at (*a*) 3 °C, (*b*) 6 °C, (*c*) 11 °C, and (*d*) 21 °C. Most of the variability for a given temperature and time after activation was among cells within a sperm activation procedure and assessment (average = 84%). On average, only 3% of the variability was among replicated activation procedures.

periment, sperm from the same semen sample and activation procedure were assessed at 30, 60, 120, 180 s (±5) after activation (Fig. 1). The entire sperm activation and assessment process was repeated completely multiple times (Fig. 1). Any replicate that deviated outside of the ±1 °C target temperature or contained sperm that were obviously drifting was discarded and was not included in the numbers reported. A minimum of three replicates was used for each fish at each temperature in the subsequent analysis (Fig. 1). These procedural replications gave very similar results (Fig. 2) and were averaged (see below) for statistical purposes.

Sperm assessment

Sperm activity was captured using a compound microscope (400× magnification) equipped with a 40× negative-phase objective and Basler camera (model A312fc) attached to a personal computer via a FireWire connector. Images were evaluated in real time using Integrated Semen Analysis System version 1.0.17 (ISAS; Projectes i Serveis R+D S.L., Valencia, Spain). The system was set to capture images at 25 frames·s^{-1}. The software estimated several potentially useful characteristics of sperm activity: average velocity on the point-to-point track followed by the cell (curvilinear velocity, VCL), average velocity of the straight line between the start and end points (linear velocity, VSL), and average velocity of the smoothed cell path (average path velocity, VAP). These are all likely correlated and one must be careful not to test the same hypothesis multiple times using different metrics.

Sperm activity was captured at the targeted times after activation and digitally stored. Each sperm recording was manually checked for quality control after the experiment was completed. Sperm tracks were removed from further analysis if the software incorrectly combined crossing tracks of multiple sperm or split the track of a single sperm or if a sperm swam out of the field of view before adequately being assessed. Accurate tracks were present for 11 075 swimming sperm. Following Tuset et al. (2008), cod sperm with VSL ≤ 4 μm·s^{-1} were not considered to be exhibiting progressive motility and were excluded (only one of the 11 075 sperm met this criterion). We did not apply the cutoff used by Rudolfsen et al. (2005, 2008), where cod sperm with VAP < 20 μm·s^{-1} and (or) VSL < 10 μm·s^{-1} were considered to be static. This would have removed 1483 sperm from our analysis in a manner that was clearly related to temperature and time after activation. Sperm that had been swimming at fast velocities often had slowed below this cutoff by 120 s at high temperatures. A total of 11 074 swimming sperm were used in subsequent analyses. Following convention of the terminology for thermal reaction norms (Angilletta 2009), we refer to sperm quality as a function of temperate as sperm performance.

Statistics

Statistics were conducted using SAS version 9.1 (SAS Institute Inc., Cary, North Carolina) and Minitab 15 (Minitab Inc., State College, Pennsylvania). Significance was set at

α = 0.05, and assumptions of parametric statistics were checked by examining model residuals.

Fish characteristics

We tried to select specific fish to eliminate fish size as a source of variability. The three cod families were compared for fish characteristics using one-way ANOVA (analysis of variance). The dependent variables were total length, body weight, Fulton's condition factor, and spermatocrit (Table 1). Bonferroni corrections were not required as families did not significantly differ in any parameter at α = 0.05.

Sperm analysis

Variance components were examined for random factors with a fully nested ANOVA (families, individuals, replicates, sperm) to determine repeatability of the experimental procedure at each time after activation for each temperature (Fig. 2). When averaged across times and temperatures, 84% of the variability in sperm swimming velocity (VCL) was due to variability among sperm cells within a procedural replicate (the activation and assessment process). This was followed by 9% for differences among individuals within a family, 4% among families, and 3% among procedural replicates (Fig. 2). Our experimental procedure for activating and documenting sperm swimming velocity was thus highly repeatable. We recommend that a similar analysis be conducted in all studies on sperm quality.

Swimming velocities of individual sperm cells were highly variable and likely non-normal in distribution. Therefore the median velocity among sperm was calculated instead of the mean within each replicated sperm activation procedure. Procedural replicates (at least three) were averaged to produce one datum for each fish (n = 12), per time after activation (n = 4), per temperature (n = 4). Different goals were addressed using different measures of sperm performance. (*i*) Faster swimming sperm are expected to be more likely to fertilize eggs, and thus sperm quality should be related to initial velocity (Rudolfsen et al. 2006). Sperm swimming trajectories are not necessarily expected to be linear, especially given that there were no female cues to potentially guide sperm (Urbach et al. 2005). Therefore swimming velocities over the actual track followed by the sperm were used as a measure of "quality" (VCL). (*ii*) To investigate how temperature and time influence variability among individual sperm cells within an ejaculate, the coefficient of variation (%) of sperm swimming velocity (VCL) was calculated within each procedural replicate (as opposed to the median) and then averaged across replicates. During the experiment, we noticed that sperm swimming "behaviour" seemed to be different at 3 °C than at the other temperatures. (*iii*) Post hoc, we sought to determine how viscosity might influence sperm propulsion using the wobble index (WOB), which is the oscillation percentage of the real track with respect to the average track (calculated as VAP/VCL by the software used). The wobble index measures the side-to-side motion of the sperm head per unit of distance traveled. High values are sperm that are swimming with less head motion, i.e., they require less tail beats to travel the same distance.

Sperm performance was analyzed using a mixed-model nested repeated-measures balanced ANOVA (Fig. 1), using Proc GLM in SAS. The main independent variables were (*i*) family (random), (*ii*) genotype (random) nested within family, (*iii*) temperature (fixed, repeated measures), and (*iv*) time (fixed, repeated measures). Appropriate interaction terms were also included and were individually assigned to obtain correct error terms for within-subjects (repeated measures) tests (Table 2).

Results

The common tank raised cod attained a body length of 45–50 cm after nearly three years of growth (Table 1). Spermatocrit was much more variable among males than were metrics of body size (coefficient of variation: total length, 5.7%; body weight, 19.6%; Fulton's condition, 7.5%; spermatocrit, 31.7%). However, the three families were not significantly different in body length, weight, condition, or spermatocrit (Table 1). Moreover, among the 12 fish used, there was no significant correlation between VCL and fish characteristics (fish length, $r = 0.083$, $p = 0.798$; body weight, $r = -0.071$, $p = 0.826$; Fulton's condition, $r = -0.471$, $p = 0.123$; spermatocrit, $r = -0.546$, $p = 0.066$).

There was phenotypic plasticity in sperm swimming velocity to temperature (Fig. 3) at all times after activation. The average response across individuals and families in Fig. 3 negates the description as a reaction norm in the strictest sense of the term (no longer the response of a specific genotype), although we will refer to it as such for continuity. The thermal reaction norms were generally dome-shaped but were more linear when sperm had been swimming for longer periods of time (Fig. 3). Thus, the relationship between swimming velocity and temperature depended on how long the sperm had been swimming (temperature × time interaction; Table 2), i.e., plasticity in plasticity (the breakdown of the interaction can be seen graphically in Fig. 3). The decline in sperm swimming velocity with time after activation occurred more rapidly at warmer temperatures (Fig. 4). At 30 s after activation, peak swimming velocities occurred at 11 °C; however, peak velocities occurred at colder temperatures if sperm had been swimming for longer periods (6 °C for 60 and 120 s and 3 °C for 180 s; Figs. 3 and 4). At the times tested, sperm generally swam faster at 3 °C than at 21 °C. Whether sperm would have swum faster at 21 °C immediately after activation is unknown (sperm were first assessed 30 s after activation).

The analysis showed a significant genotype × environment interaction (genetic differences in phenotypic plasticity). Unexpectedly, thermal reaction norms of sperm swimming velocity differed more among individuals within a family than among families (Fig. 2; Table 2). This result is based on a nested analysis indicating where most of the variation exists (see Discussion). Reaction norms are shown for each of the 12 individuals (Fig. 5). The full analysis accounts for repeated measures of temperature and time after activation, families, individuals within families, and appropriate interactions (Table 2). There were no overall statistically significant differences among the three families or in how they responded to temperature and time (Table 2). However, there were substantial differences among individuals and this depended on both time after activation and tem-

Table 2. Mixed-model nested repeated-measures balanced analysis of variance (ANOVA) for comparing sperm performance of Atlantic cod (*Gadus morhua*).

				Sperm swimming performance					
				Sperm velocity, median VCL ($\mu m \cdot s^{-1}$)		Sperm variability, coefficient of variation VCL (%)		Sperm wobble, VAP/VCL (%)	
Source	Term	df	Error	*F*	p^a	*F*	p^a	*F*	p^a
1	Family	2	2	2.76	0.116	2.00	0.191	1.60	0.254
2	Genotype (Family)	9	11	28.28	<0.001	7.11	<0.001	7.57	<0.001
3	Temp	3	5	86.25	<0.001	6.91	0.001	123.23	<0.001
4	Family × Temp	6	5	0.77	0.603	2.01	0.099	0.55	0.767
5	Temp × Genotype (Family)	27	11	2.76	<0.001	2.60	0.001	2.39	0.001
6	Time	3	8	60.63	<0.001	5.57	0.004	44.41	<0.001
7	Family × Time	6	8	1.00	0.448	0.62	0.709	1.80	0.138
8	Time × Genotype (Family)	27	11	3.20	<0.001	1.98	0.010	1.32	0.173
9	Temp × Time	9	11	6.90	<0.001	1.35	0.223	6.01	<0.001
10	Family × Temp × Time	18	11	0.89	0.587	0.83	0.657	1.18	0.301
11	Error	81							
	Total	191							

Note: Source is a code for the different terms; df is degrees of freedom; error refers to which source is used in the denominator of the *F* test; and Temp is temperature. Genotype refers to an individual fish and is used to match standard convention for assessing G × E interactions. The term Temp × Time × Genotype (Family) is not included in the model; this appears as error (source 11) and is the correct error term for testing five of the other terms. Sperm velocity (VCL) is the mean among procedural replicates of the median among sperm cells within a procedural replicate. Sperm variability is the arsine-transformed mean among procedural replicates of the coefficient of variation in velocity (VCL) among sperm cells within a procedural replicate. Wobble is the oscillation index (high values have less wobble) and has been arsine-transformed.

[a] The models explained 96.1%, 82.0%, and 94.3% of the variance, respectively.

Fig. 3. Average reaction norms of sperm curvilinear swimming velocity (VCL) at four test temperatures. Swimming velocities at assigned elapsed time periods after sperm activation are shown as different symbols: 30 s (○), 60 s (△), 120 s (□), and 180 s (▽). Values shown are means among individuals and families (*N* = 12 fish). Note that thermal reaction norms are more linear for sperm that have been swimming longer.

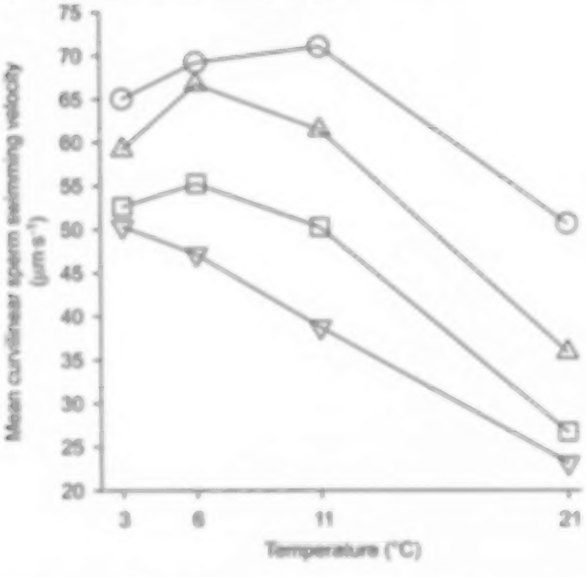
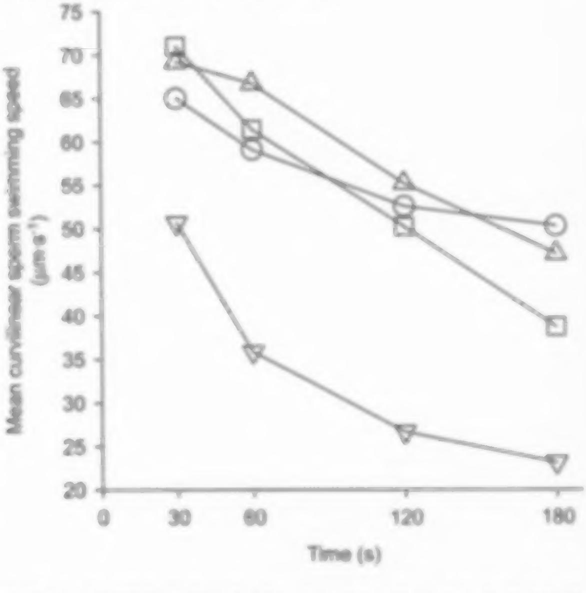
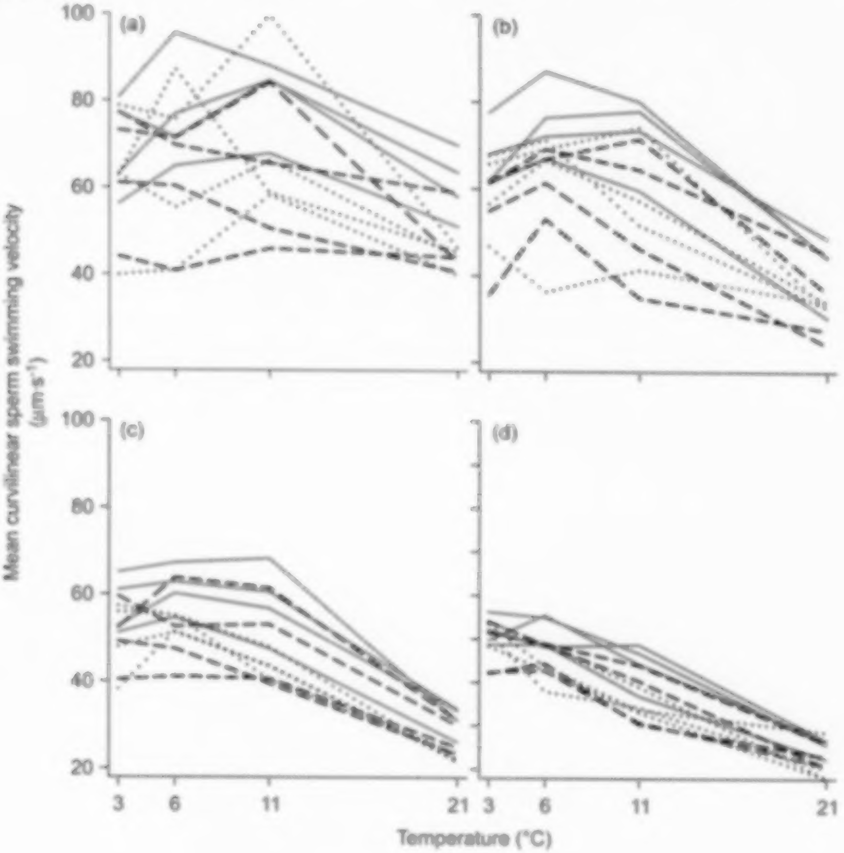


Fig. 4. Average sperm swimming velocity (VCL) through time for each of the different temperatures. Swimming velocities at the different temperatures are shown as different symbols: 3 °C (○), 6 °C (△), 11 °C (□), and 21 °C (▽). Values shown are means among individuals and families (*N* = 12 fish).



perature (significant Temp × Genotype (Family) interaction; Table 2). For example, at 30 s after activation, swimming velocities of three individuals from family B increased from 6 °C to 11 °C but sharply decreased for the other individual, whereas temperature had much less affect on sperm from three of the four individuals from family C (Fig. 5). Variability among individuals was highest at 3 °C and lowest at 21 °C.

Fig. 5. Reaction norms of sperm swimming velocity at four test temperatures. Swimming velocities at assigned elapsed time periods since sperm activation are shown as different panels: (*a*) 30 s, (*b*) 60 s, (*c*) 120 s, and (*d*) 180 s. Values shown are individual genotype averages among procedural replicates. Family A, shaded continuous lines; family B, dotted lines; family C, broken lines. There is a significant genotype × environment interaction.



Individual sperm from the same animal (i.e., genotype) appear to have responded differently to environmental variation. The coefficient of variation in sperm swimming velocity was affected by time after activation and temperature but not their interaction (Fig. 6; Table 2). Variability among sperm cells within ejaculates did not significantly differ among families but did vary among individuals, and this depended on both time after activation and temperature (Table 2).

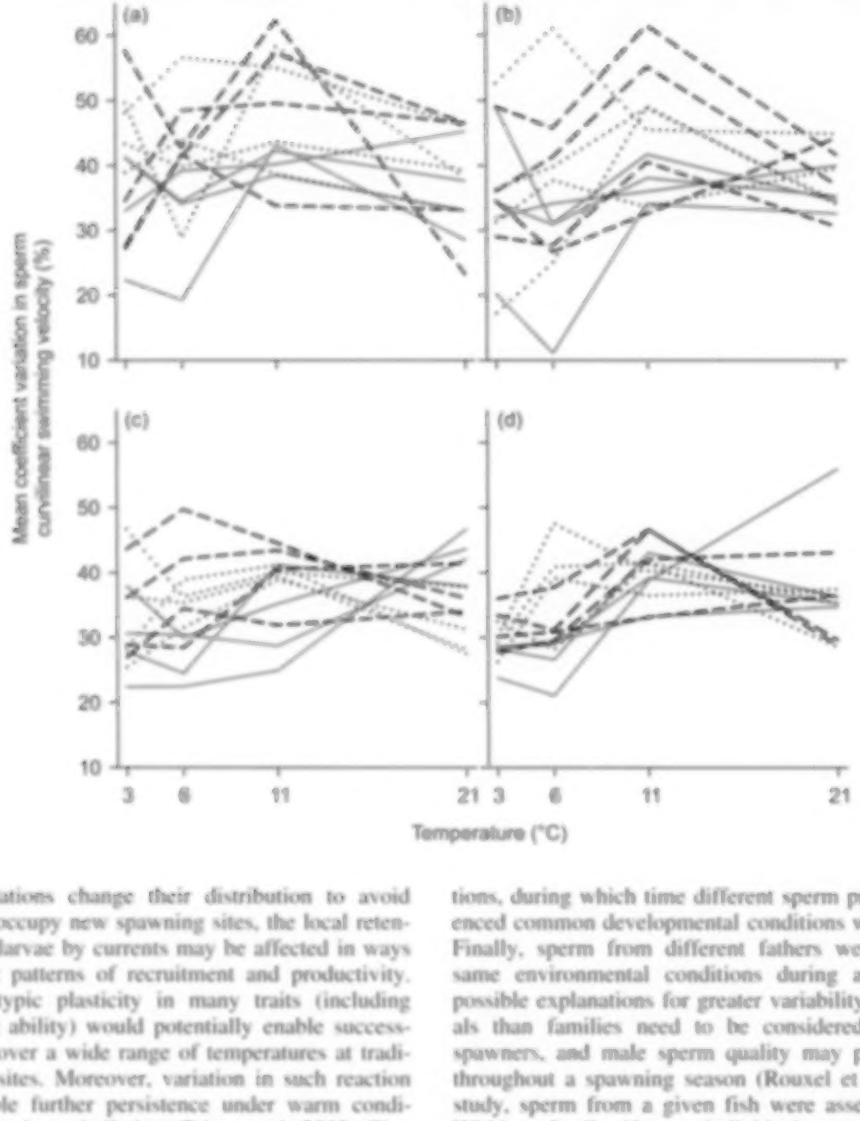
Wobble was significantly influenced by both temperature and time after activation and their interaction (Table 2; the nature of the interaction can be seen in Fig. 7). Sperm exhibited much more side-to-side swimming motion at 3 °C than at the other temperatures, but there was significant variability among individuals (Table 2; Fig. 7). There was less of an affect from time than temperature, but on average, sperm that had been swimming for longer periods tended to show slightly more side-to-side motion.

Discussion

Atlantic cod sperm exhibited phenotypic plasticity in swimming performance across temperatures; however, the description of the reaction norm is dramatically different depending on how long sperm had been swimming when assessed. Thus, the shape of phenotypic plasticity to one environment (temperature) is dependent on another (time). There was a statistically significant G × E interaction at one hierarchical level, but not at another. Unexpectedly, differences in thermal reaction norms of sperm performance were greater among individuals within a family than among families (discussed below). To our knowledge, this is the first study on sperm to describe performance as a reaction norm to variable environments. Additionally, we found no published study on a sexually reproducing animal that explicitly stated that reaction norms were documented for individual genotypes, which could be due to no previous research or a lack of use of the terminology.

Selection can act on genotypic differences in phenotypic plasticity, and therefore populations may evolve new reaction norms under changing conditions (Gotthard et al. 1995). Current climate trends are predicted to produce increases in ocean temperatures into the foreseeable future, and although cod spawn seasonally at specific water temperatures, thermal limits for successful reproduction are un-

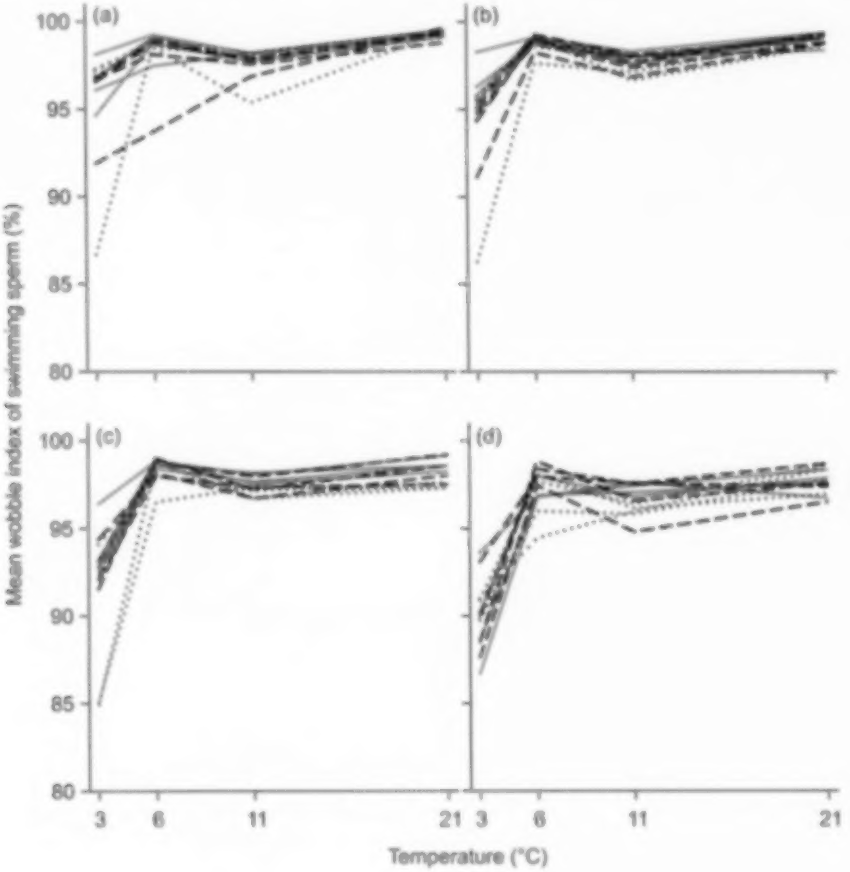
Fig. 6. Mean (among replicates) coefficient of variation of sperm curvilinear swimming velocity (within replicates) at four test temperatures. Swimming velocities at assigned elapsed time periods since sperm activation are shown as different panels: (*a*) 30 s, (*b*) 60 s, (*c*) 120 s, and (*d*) 180 s. Values shown are individual genotype averages among procedural replicates. Family A, shaded continuous lines; family B, dotted lines; family C, broken lines. There is a significant genotype × environment interaction.



known. If populations change their distribution to avoid warm water and occupy new spawning sites, the local retention of eggs and larvae by currents may be affected in ways that alter current patterns of recruitment and productivity. However, phenotypic plasticity in many traits (including sperm swimming ability) would potentially enable successful reproduction over a wide range of temperatures at traditional spawning sites. Moreover, variation in such reaction norms may enable further persistence under warm conditions through genetic assimilation (Price et al. 2003; Ghalambor et al. 2007).

Our experiment was designed to investigate genetic differences in phenotypic plasticity, under controlled environments, at two levels: within and among families. Unexpectedly, our results show greater variability among individuals within a family than among families. The reason for this is unknown. In our opinion, our experiment had more control than would be typical with a species such as cod. For example, the source fish were collected at the same time and place and experienced the same captive conditions before being used in artificial fertilizations. The F_1 offspring were raised for three years under common conditions, during which time different sperm presumably experienced common developmental conditions within their father. Finally, sperm from different fathers were treated to the same environmental conditions during assessment. Some possible explanations for greater variability among individuals than families need to be considered. Cod are batch spawners, and male sperm quality may potentially change throughout a spawning season (Rouxel et al. 2008). In our study, sperm from a given fish were assessed on one day. Within a family, if some individuals were at peak seasonal performance and some others at pre- or post-peak performance, individual variability in sperm quality associated with spawn timing may have been enough to mask relatively higher sperm variability among families in the nested analysis (although it seems more likely that individuals within a family would be synchronized in their spawning cycles than fish from different families). Additionally, sperm from all fish could not be assessed at the same time, so we chose to control most carefully for temperature and family comparisons. As a result, the different individuals within a family were not assessed on the same day. However, there is no obvious reason for this to have introduced variability. For ex-

Fig. 7. Reaction norms of sperm swimming behaviour at four test temperatures. The mean wobble index is plotted on the *y* axis; note that higher values are sperm swimming with less side-to-side head motion. The different time periods since sperm activation are shown as different panels: (*a*) 30 s, (*b*) 60 s, (*c*) 120 s, and (*d*) 180 s. Values shown are individual genotype averages among procedural replicates. Family A, shaded continuous lines; family B, dotted lines; family C, broken lines. There is a significant genotype × environment interaction.



ample, the water used in sperm activation on all days was obtained from a common container that was kept in a cold room at 6 °C, and air was bubbled into the activation medium to ensure oxygen saturation.

Temperature influences fish swimming performance through both physiological and physical mechanisms. High temperature may increase metabolism but is strongly inversely related to viscosity. The swimming behaviour of ~55 cm African lungfish (*Protopterus annectens*) was affected at higher viscosities (mud) because less distance was travelled per tail beat, i.e., the stride length decreased (Horner and Jayne 2008). At the millimetre scale, Fuiman and Batty (1997) separated mechanical from physiological effects of temperature on the swimming behaviour of larval herring (*Clupea harengus*) and found that smaller individuals were much more susceptible to the effects of viscosity than larger larvae. Because of their extremely small size, viscosity is expected to have a large effect on sperm. Kupriyanova and Havenhand (2005) showed that about half of the decline in polychaete sperm swimming velocity with temperature could be attributed to viscosity. In our study, sperm generally swam with more side-to-side motion at the lowest temperature (highest viscosity), but there was also more variability among individual fish at 3 °C. Presumably, given reductions in stride length through higher viscosity water, sperm must use more tail beats to cover the same distance, which results in more side-to-side motion over the swimming track. However, we cannot separate physiological and mechanical effects of temperature. Moreover, the kinematic viscosity for seawater at the test temperatures would be as follows: at 3 °C, $1.66 \times 10^{-6}\ \mathrm{m^2 \cdot s^{-1}}$; 6 °C, $1.52 \times 10^{-6}\ \mathrm{m^2 \cdot s^{-1}}$; 11 °C, $1.32 \times 10^{-6}\ \mathrm{m^2 \cdot s^{-1}}$; and 21 °C, $1.03 \times 10^{-6}\ \mathrm{m^2 \cdot s^{-1}}$. Thus the proportional change in wobble at the lowest temperature cannot be explained by an equivalent proportional change in viscosity.

The production of novel phenotypes may be expected in extreme or stressful environments (Ghalambor et al. 2007) because of the release of cryptic genetic variation (Queitsch et al. 2002; Rutherford 2000, 2003). We tested this hypothesis with 11 074 sperm from 12 individual Atlantic cod. If novel phenotypes are produced under extreme environments, we would predict higher variability among individual fish in those situations. Variability among individual fish in sperm swimming velocity and wobble tended to be greatest at the coldest temperature but was most uniform at the highest temperature, and thus, the test is inconclusive. At 3 °C, our coldest test temperature is closer to preferred cod spawning temperatures than 21 °C. Such results might be explained by sperm "burning out" at 21 °C before our first assessment at 30 s after activation, which may or may not be related to the use of a limited supply of ATP (Burness et al. 2005). Further research should be directed to testing this hypothesis.

Our study builds on several others that have used common-garden experiments to investigate reaction norms of Atlantic cod in relation to temperature. When two populations were grown under similar conditions, the population from the higher latitude had faster growth rates, but this was independent of temperature (Purchase and Brown 2000). Purchase and Brown (2001) showed genetic differences among populations in how relative liver weight (major energy store) responded to changing water temperatures. Research on three populations of Northwest Atlantic cod showed differences in phenotypic plasticity in body shape to constant rearing temperatures and food supply (Marcil et al. 2006). Hutchings et al. (2007) found genetic differences in life history reaction norms of cod, where warm-water populations were more sensitive to food and cold-water populations were more sensitive to temperature. This growing body of literature highlights important genetic differences among populations; however, our study is the first to investigate individual variation in phenotypic plasticity.

Two types of studies have investigated the influence of temperature on sperm. One has researched the effect of maintaining adults at different temperatures before the collection of semen and then comparing sperm at common temperatures. Shrimp (*Litopenaeus vannamei*) kept at lower temperatures produced more sperm and had higher proportions of normal sperm than those at kept at high temperatures (Perez-Velazquez et al. 2001). Carp (*Cyprinus carpio*) kept at cold and warm temperatures produced sperm with different chemical composition, but they did not differ in proportion motile or in how long sperm remained motile (Emri et al. 1998). Other studies have compared sperm performance under different temperatures of the sperm activating medium. Vladić and Järvi (1997) investigated sperm longevity in two species of fish, although they did not place the results into the context of reaction norms. Sperm swam for longer time periods in colder water for both species, but the response to temperature was more sensitive for Atlantic salmon (*Salmo salar*) than for brown trout (*Salmo trutta*). See Alavi and Cosson (2005) for a review of both types of studies for three groups of fishes. Our study is unique in that we compare variability within and among families in different environments.

Our research contributes to a growing body of literature on cod semen. In most fish species that have been investigated (largely freshwater species), sperm swim for short periods of time, with longevity generally being less than 1 min. In contrast, Atlantic cod sperm swim for long periods and can achieve up to 50% fertilization success after 1 h under artificial conditions (Trippel and Morgan 1994). However, because of finite energy stores, faster-swimming sperm are predicted to swim for shorter periods of time (shorter longevity), and thus longevity may be inversely related to fitness within species, especially those under intense sperm competition such as cod. Whether the longevity of cod sperm is adaptive under natural situations is unknown. The proportion of sperm that are motile quickly declines after activation (Cosson et al. 2008). However, percent motility has repeatedly been shown to have no effect on cod fertilization ability (Trippel and Neilson 1992; Rakitin et al. 1999*a*; Rudolfsen et al. 2008). On the other hand, higher sperm densities increase fertilization success, both when artificially pipetted (Butts et al. 2009) and when occurring naturally in variable ejaculates (spermatocrit; Rakitin et al. 1999*a*). Faster-swimming sperm likely fertilize more eggs (Rudolfsen et al. 2008; Skjæraasen et al. 2009), although this is not certain (Trippel and Neilson 1992; Rudolfsen et al. 2005), and evaluation of swimming speed is variable depending on the presence of maternal cues (Litvak and Trippel 1998). Sperm quality changes through the spawning season (Rouxel et al. 2008) and seems to be influenced by male condition (relative weight) but not absolute size (Rakitin et al. 1999*a*; Tuset et al. 2008). Percentage of motile cells and swimming speeds are influenced by salinity (Litvak and Trippel 1998).

Here we demonstrated that temperature influences sperm swimming velocity but that the magnitude and direction of the response depends on individual variability and the time after activation, which might be of practical importance to aquaculture operations. This also indicates that caution should be taken when drawing conclusions about potential lack of genetic differences in phenotypic plasticity in studies that use limited numbers of environments or genotypes. Additionally, the reason for the relatively high amount of variability among sperm cells within an ejaculate (as compared with among replicates, individuals, families) is unknown but may help explain why sperm quality is hard to predict from fish characteristics. As a result, we recommend that the variability breakdown presented in Fig. 2 be shown in all studies on sperm quality. Our cod had been maintained at the same temperature, and thus the effect of temperature on sperm performance of fish that have been acclimated to different conditions remains unknown.

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When can we reliably estimate the productivity of fish stocks?

Paul B. Conn, Erik H. Williams, and Kyle W. Shertzer

Abstract: In modern fishery stock assessments, the productivity of exploited stocks is frequently summarized by a scale-invariant "steepness" parameter. This parameter, which describes the slope of the spawner–recruit curve, determines resilience of a stock to exploitation and is highly influential when estimating maximum sustainable yield. In this study, we examined conditions under which steepness can be estimated reliably. We applied a statistical catch-age model to data that were simulated over a broad range of stock characteristics and exploitation patterns and found that steepness is often estimated at its upper bound regardless of underlying productivity. The ability to estimate steepness reliably was most dependent on the true value of steepness, the exploitation history of the stock, natural mortality, duration of the time series, and quality of an index of abundance; this ability was relatively unaffected by levels of stochasticity in recruitment and sampling intensity of age compositions. We further explored the method of inverse prediction to improve estimates of steepness and conclude that this approach holds promise. We illustrate the utility of simulation and inverse prediction methods with two fish stocks located off the southeastern United States, greater amberjack (*Seriola dumerili*) and gag grouper (*Mycteroperca microlepsis*).

Résumé : Dans les évaluations modernes des stocks, la productivité du stock exploité est souvent résumée par un paramètre d'« inclinaison » qui est invariable à toutes les échelles. Ce paramètre, qui décrit la pente de la courbe reproducteurs–recrues, détermine la résilience d'un stock à l'exploitation et est très important dans la détermination du rendement maximum durable. Notre étude examine les conditions dans lesquelles l'inclinaison peut être estimée de façon fiable. Nous avons utilisé un modèle statistique des captures en fonction de l'âge avec des données simulées présentant un vaste éventail de caractéristiques des stocks et de patrons d'exploitation et avons découvert que l'inclinaison est souvent estimée à sa limite supérieure, quelle que soit la productivité sous-jacente. La possibilité d'estimer l'inclinaison de manière fiable dépend surtout de la valeur réelle de l'inclinaison, de l'histoire d'exploitation du stock, de la mortalité naturelle, de la durée de la série chronologique et de la qualité de l'indice d'abondance; cette possibilité est relativement peu affectée par les niveaux de stochasticité dans le recrutement et l'intensité de l'échantillonnage de la composition en âge. Nous avons éprouvé en plus la méthode de prédiction inverse pour améliorer les estimations de l'inclinaison et concluons que la méthode est prometteuse. Nous illustrons l'utilité de la simulation et de la prédiction inverse à l'aide de deux stocks de poissons du large du sud-est des États-Unis, ceux de la grande sériole (*Seriola dumerili*) et de la badèche baillou (*Mycteroperca microlepis*).

[Traduit par la Rédaction]

Introduction

Estimating the parameters of a spawner–recruit function is one of the most vexing challenges in fisheries stock assessment. Ultimately, these parameters control the productivity and resilience of modeled fish stocks and thus are fundamental for predicting a stock's response to exploitation and for calculating management benchmarks. In practice, several factors act in unison to limit our knowledge of spawner–recruit relationships. This list includes process and observation errors, errors-in-variables, nonstationarity, time series bias, and — perhaps the most serious — lack of contrast in spawning biomass (Hilborn and Walters 1992; Quinn and Deriso 1999).

When virgin abundance is regulated by food or habitat (as opposed to cannibalism, for instance), the Beverton–Holt functional form satisfies desirable properties of spawner–recruit relationships (Ricker 1975). Assessments increasingly use a version of the Beverton–Holt model that is parameterized in terms of a "steepness" parameter h (Mace and Doonan 1988) that quantifies the degree of density dependence in recruitment. In this parameterization, steepness is bounded, $0.2 \le h \le 1.0$. Researchers often prefer this version because h is independent of scale (Shertzer et al. 2008) and thus affords comparison among stocks through, for example, meta-analysis, whereby data from similar stocks can be shared hierarchically (Liermann and Hilborn 1997; Myers et al. 1999; Michielsens and McAllister 2004).

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In this article, we address the question, "Under what conditions can we estimate steepness reliably?" We ask this both in a general sense and for specific populations. For the general case, our strategy is first to simulate data under a wide variety of life history strategies and management regimes and then to estimate *h* using statistical catch-age analysis (Fournier and Archibald 1982; Deriso et al. 1985; Methot 1990). We then relate variation in estimated steepness to simulation input variables. This approach is similar in spirit to previous simulation studies (e.g., Ianelli 2002; Yin and Sampson 2004; Magnusson and Hilborn 2007) but focuses explicitly on steepness.

Previous studies reached mixed conclusions about the impact of variables likely to affect *h* or management benchmarks derived from *h*. For instance, when addressing steepness in particular, Magnusson and Hilborn (2007) emphasized the importance of having age composition or relative abundance index data at low stock sizes. More recently, Haltuch et al. (2008) emphasized the length of catch time series and recruitment variability and found that variables related to sampling intensity (e.g., number of age compositions, precision of index values) were less important. Both of those studies had a substantial number of simulations in which *h* was estimated at its upper bound of 1.0. Such an estimate implies that recruitment is unrelated to spawning biomass, providing a highly optimistic picture of the resilience of the stock. Furthermore, high steepness shortens estimated rebuilding schedules of depleted stocks, because expected recruitment remains high even at the lowest stock sizes.

In addition to a large-scale simulation study, we also consider two case studies — greater amberjack (*Seriola dumerili*; GAJ) and gag grouper (*Mycteroperca microlepsis*; GAG) off the southeastern United States. In recent stock assessments of these species, the point estimate of steepness was at its upper bound for gag (SouthEast Data, Assessment, and Review (SEDAR) 2006), but not for greater amberjack (SEDAR 2007). Both assessments used a bootstrapping procedure to approximate uncertainty around point estimates, and for both stocks, a large number of bootstrap iterations resulted in steepness estimated at its upper bound. This situation is clearly undesirable if the high steepness values reflect poor estimation rather than actual stock productivity. We try to assess the reliability of estimation using stock-specific simulations, and in particular, we explore the potential of inverse prediction (cf., Osborne 1991) to refine point estimates of steepness by calibrating them to known true values used in simulations.

This paper is structured as follows. First, we review relevant spawner–recruit theory. Second, we provide a brief summary of inverse prediction methods and describe how they can be useful in fisheries applications. We then describe the methods that we used for large-scale simulations, as well as methods for analyzing simulation results. Descriptions of the two case studies follow, including details of how inverse prediction methods were used to refine steepness estimates. After presenting results, we discuss implications of our work for stock assessment and benchmark determinations.

Materials and methods

Steepness

The Beverton–Holt spawner–recruit function (Beverton and Holt 1957) has a rich history in fish ecology for predicting the number of recruits to a population given a level of spawning biomass. In modern stock assessment, a common parameterization of this model is

$$R = \frac{0.8R_0hS}{0.2\Phi_0R_0(1-h)+(h-0.2)S}$$

where R is the number of new recruits, S is spawning biomass, h is steepness, R_0 is mean "virgin" recruitment (i.e., that expected prior to exploitation), and Φ_0 is unfished spawning biomass per recruit (Mace and Doonan 1988). The formal definition of h under this parameterization is "the proportion of R_0 produced by 20% of S_0," where S_0 is the theoretical spawning biomass from an unfished stock (Shertzer et al. 2008). If natural mortality, growth, and maturity schedules are known, Φ_0 can be computed as

$$\Phi_0 = \sum_{a=1}^{A} W_a\mu_a\exp[-(a-1)M]$$

where A is the maximum age, W_a is weight at age a, μ_a is the proportion mature at age a, and M is the instantaneous natural mortality rate.

Given a time series of spawner–recruit data, the parameters R_0 and h may then be estimated using a method such as maximum likelihood. A lognormal error structure is often used to describe recruitment deviations (Chen 2004), and in modern stock assessment models, the time series of S and R are often estimated at the same time as the spawner–recruit parameters. If $h = 0.2$ (its lower bound), recruitment is directly proportional to spawning biomass. If $h = 1.0$ (its upper bound), recruitment is independent of spawning biomass. In the latter case, overfishing has no effect on recruitment, although it may reduce yield if fish are routinely being harvested before reaching an "optimal" size.

Inverse prediction

Fishery biologists increasingly use statistical catch-age (SCA) analysis to perform stock assessments. Model fitting requires optimizing a compound objective function that often includes penalties and weights to control various aspects of model fit. These penalties and weights make asymptotic variance estimates unreliable. Variance estimates also break down when bounded parameters lie on a boundary (e.g., 1.0 for steepness). We suggest that inverse prediction may be a viable approach in such cases.

Inverse prediction has been used following controlled calibration experiments (see, e.g., Brown 1982; Osborne 1991; Benton et al. 2003). In typical applications, there is a variable of interest (say, X) that is difficult or expensive to measure, and another that is much easier to measure (call this variable Y). A calibration experiment is conducted to determine the relationship between X and Y, and this relationship is summarized in a model such as

$$Y = f(X, \boldsymbol{\theta}) + \varepsilon$$

where $f(\cdot)$ is a function chosen by the investigator, $\boldsymbol{\theta}$ is an unknown parameter vector, and ε is an error term. Once this relationship has been quantified, interest typically focuses on new observations Y_0, where X_0 is unknown. That is, given a new response variable that is easy to measure (Y_0), what can one say about the unmeasured predictor variable?

One of the more flexible approaches to inverse prediction uses Bayesian inference to quantify uncertainty in X_0 given a new observation Y_0. Following Racine-Poon (1988), inference about X_0 can be made with this approach using posterior simulation where

$$(1)\qquad [X_0, \boldsymbol{\theta} | Y_0, \boldsymbol{X}, \boldsymbol{Y}] \propto [Y_0 | X_0, \boldsymbol{\theta}][X_0][\boldsymbol{X}, \boldsymbol{Y} | \boldsymbol{\theta}][\boldsymbol{\theta}]$$

Here $[A]$ denotes the probability density function of a random variable A, $[A|B]$ denotes the conditional distribution of A given B, and $\boldsymbol{X}$ and $\boldsymbol{Y}$ are vectors of predictors and responses from the calibration experiment.

How is this framework useful for improving estimates of steepness from stock assessment? We propose that a simulation experiment be used to quantify the relationship between true and estimated steepness values, and that this relationship be used to infer a plausible distribution of steepness given a single value estimated from an SCA analysis on a given fish stock (cf., Fig. 1). This approach treats the SCA analysis as a "black box" in that a solution is reached but it is unnecessary for the resultant estimators to possess known theoretical properties (description of technical detail is deferred to Appendix A).

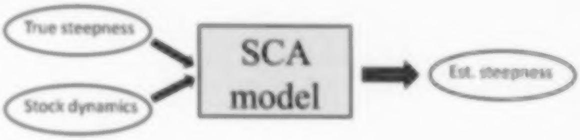
General simulation study

To investigate the reliability of steepness estimates produced by modern stock assessment models, we conducted a Monte Carlo simulation study. Our strategy was to consider variability in stock dynamics and data collection wherever we thought that such variability might influence estimation of steepness; in other cases, we fixed values across simulations or set them based on theory of life history invariants (e.g., Charnov 1993). Virtual stocks varied by natural mortality, productivity, exploitation pattern, and level of process error and were monitored for different lengths of time and with different levels of observation error (Table 1).

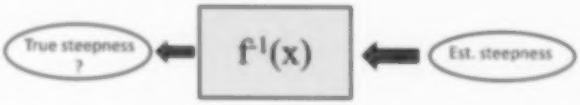
Four possible harvest regimes ($F_{\text{type}} \in 1, 2, 3, 4$) were considered, with fishing rates defined relative to F_{MSY}, the fishing rate that provides maximum sustainable yield (MSY). In regimes 1 and 2, mortality rates were constant over time at low ($F/F_{\text{MSY}} = 0.5$) or high ($F/F_{\text{MSY}} = 2.0$) levels, respectively. In regime 3, mortality rates increased linearly from $F/F_{\text{MSY}} = 0.5$ at the beginning of the study to $F/F_{\text{MSY}} = 2.0$ at the middle of the time series, before declining back to $F/F_{\text{MSY}} = 0.5$ at the end of the study. This scenario ("two-way trip") mimics the situation in which exploitation increases until resource managers realize that overfishing is occurring and then act to decrease fishing pressure. Hilborn (1979) showed that rebuilding periods were important for estimating productivity with biomass-dynamic models, although their importance for modern statistical catch-age models is perhaps more equivocal (Magnusson and Hilborn 2007). Regime 4 consisted of a "one-way trip" whereby fishing mortality rates increased linearly from $F/F_{\text{MSY}} = 0.5$ at the beginning of the study to $F/F_{\text{MSY}} = 2.0$ at the end of the study. In all cases, initial stock status was set at equilibrium values associated with the fishing mortality rate in year one.

Fig. 1. A visual depiction of inverse prediction as applied to steepness in stock assessment. The first step (*a*) consists of a calibration experiment in which simulated fisheries data are passed into a statistical catch-age analysis to estimate steepness. In the second step (*b*), it is assumed that the same statistical catch-age model from step *a* is used to estimate steepness for a given stock of interest. A functional relationship between true and estimated steepness from step *a* is then used to predict the true value of steepness given the SCA estimate. In practice, the second step can be accomplished by sampling eq. 1.

(a) *Calibration experiment*



(b) *Inverse prediction*



When simulating data, we assumed that stock dynamics were governed by a set of age-structured equations subject to lognormal process error in the Beverton–Holt recruitment relationship (Table 2). Selectivity and maturity schedules followed a logistic function, and harvesting and natural mortality processes were continuous with piecewise constant hazard rates (cf., Cox and Oakes 1984). The stock was exploited by a single fishery, which provided the assessment with three data sets for model fitting: total annual landings, samples of age compositions of landings, and an index of abundance derived from catch per unit effort. We assumed that observed landings and the index of abundance were subject to lognormal error and that observed age compositions were multinomially distributed (Table 2). All of these formulations are standard in models of fish dynamics (Quinn and Deriso 1999).

Estimation

We used two different estimation procedures on each data set. We started by fitting an SCA model to the data (Table 3). 3), which was similar in structure to stock synthesis models (Methot 1989; 1990) and implemented in AD Model Builder software (Otter Research Ltd. 2004). Among other things, this procedure produced estimates of historical stock and recruit time series, as well as steepness. Spawner–recruit deviations were loosely constrained to follow a Beverton–Holt spawner–recruit model (Table 2). Because the constraint was loose, we found little difference between estimates of spawner and recruit time series using this approach and one with no defined spawner–recruit relationship. We denote estimates of steepness from AD Model Builder as $\hat{h}^I$ to indicate that they were estimated internally in the stock

Table 1. Definitions of input variables with ranges used for general fish stock simulations. Each input variable was randomly sampled 10 000 times using a uniform distribution, with three replicate simulations performed at each such design point.

Parameter	Range	Definition
Y	(10, 45)	Years of data
M	(0.1, 0.4)	Instantaneous natural mortality rate
h	(0.21, 1.0)	Steepness
σ_R	(0.2, 0.8)	Standard deviation for multiplicative lognormal random errors applied to the spawner–recruit function
σ_I	(0.0, 0.5)	Standard deviation of abundance index
n	(50, 1000)	Number of fish aged annually
F_{type}	{1, 2, 3, 4}	An index describing which time series of fishing mortality rates were used
A	$1+\sum_{a=1}^{\infty} I_{\exp(-aM)>0.02}(a)$	Age when "plus" group begins
α	0.000001 (fixed)	Length–weight constant ($w = \alpha l^{\beta}$)
β	3.0 (fixed)	Length–weight exponent ($w = \alpha l^{\beta}$)
l_{∞}	1000 (fixed)	von Bertalanffy asymptotic size
t_0	–0.5 (fixed)	Age at size zero for von Bertalanffy growth model
K	$= M/1.65$	von Bertalanffy growth rate, parameterized using life history invariant of Charnov (1993)
a_{50}	$= \frac{1}{K}\log\left(\frac{3K+M}{M}\right)$	Age at 50% maturity and selectivity (Beverton 1992)
η	1.0 (fixed)	Slope parameter for logistic selectivity and maturity functions
R_0	100 000 (fixed)	Asymptotic recruitment when $F = 0$
q	0.000001 (fixed)	Catchability associated with abundance index

assessment model. Second, we used the predicted stock and recruit time series to estimate steepness externally via maximum likelihood. Following Dorn (2002), this approach involved maximizing a joint lognormal probability density function for recruitment with a bias correction:

$$\prod_{y}\frac{1}{R_y\sqrt{2\pi}\,\sigma_R}\exp\left[-\frac{1}{2\sigma_R^2}\left(\log(R_y)-\log(\tilde{R}_y)+\sigma_R^2/2\right)^2\right]$$

where R_y denotes "observed" recruitment (i.e., output of the assessment model), and $\tilde{R}_y$ denotes recruitment as predicted by the Beverton–Holt model. We denote estimates of steepness from this approach as $\hat{h}^E$ to indicate that they were estimated externally. Estimates $\hat{h}^I$ and $\hat{h}^E$ were evaluated using median absolute relative error (MARE), a common metric for assessing estimator performance in fishery simulation studies (e.g., Prager 2002; Punt 2003; Wilberg and Bence 2006).

For simplicity, natural mortality was treated as a known constant in the assessment model and was set to its true value. Similarly, we assumed that the parameters describing growth and age–length conversions were known without error, and we supplied the assessment model with the correct standard deviations for the error in landings and the population index. Thus, the assessment model was provided a favorable scenario for estimating steepness.

Analysis of simulation performance data

Preliminary analysis suggested a bimodal distribution for estimated steepness (see, e.g., Fig. 2). In particular, steepness was estimated either at its upper bound of 1.0 or imprecisely but centered on or near the true value. We thus conceptualized $\hat{h}$ as being drawn from a one-inflated and truncated normal distribution. Written as a finite mixture distribution and taking into consideration that $\hat{h} \in (0.2, 1.0)$, the probability density function (pdf) for $\hat{h}$ is then

$$(2)\qquad f(\hat{h}|\mu_h,\sigma_h,\pi) = \pi + (1-\pi)\frac{\mathcal{N}(\hat{h}|\mu_h,\sigma_h^2)}{\int_{0.2}^{1.0}\mathcal{N}(\hat{h}|\mu_h,\sigma_h^2)d\hat{h}}$$

Here, $\mathcal{N}(\mu_h\sigma_h^2)$ denotes the normal pdf, and π gives the probability that h is estimated at its upper bound. Our interest focused on trying to identify important predictors of π (i.e., what factors influence steepness being estimated at its upper bound?) and σ_h (i.e., given that steepness is not estimated at 1.0, what factors influence precision of $\hat{h}$?).

We initially found that estimating all parameters simultaneously was computationally prohibitive. Instead, we fit the model using a two-step process. First, π was estimated using a generalized additive model (GAM; Hastie and Tibshirani 1999) where the binary response variable was whether $\hat{h}$ was estimated to be greater than 0.998. In this case, we used the function "gam" in the R programming language (R Development Core Team 2007), specifying cubic smoothing splines for all continuous variables. Second, conditional on $\hat{h} \leq 0.998$, we estimated σ_h using generalized linear models (GLMs; McCullagh and Nelder 1989) (a GAM was not used because the truncated normal distribution is not a standard error distribution). In this case, we assumed that steepness was being estimated without bias (that is, $\mu_h = h$), which indeed seemed to be the case when exploring results visually. We used the function "nlminb" for maximum likelihood inference on the constrained normal distribution and used a logit link function on σ to constrain its values to reasonable levels and to promote numerical stability.

Table 2. Formulation of population dynamics model and description of how data were generated.

Quantity	Symbol	Description or definition
Modeled quantities		
Weight at age	w_a	$w_a = al_a^b$
Maturity	m_a	$m_a = \frac{1}{1+\exp[-\eta(a-a_{50})]}$
Fishery selectivity*	s_a	Data were generated with $s_a = m_a$, but logistic selectivity parameters were estimated within the SCA analysis
Fishery mortality rate*	F_y	Annual fishing mortality
Fishing mortality rate at age	$F_{a,y}$	$F_{a,y} = s_{a,y}F_y$, where F_y is the fully selected fishing mortality rate
Total mortality rate at age	$Z_{a,y}$	$Z_{a,y} = M + F_{a,y}$
Catchability coefficient*	q	Set to 0.00001 when generating data; estimated in the SCA analysis
Abundance at age*	$N_{a,y}$	$N_{1,1} = \frac{R_0(4h\phi_F - \phi_0(1-h))}{(5h-1)\phi_F}\exp(\varepsilon_0^R)$ $N_{a+1,1} = N_{a,1}\exp(-Z_{a,1}) \quad \forall a \in (1, \ldots, A-1)$ $N_{A,1} = N_{A-1,1}\frac{\exp(-Z_{A-1,1})}{1-\exp(-Z_{A,1})}$ $N_{0,y+1} = \frac{0.8R_0hS_y}{0.2\phi_0R_0(1-h)+(h-0.2)S_y}\exp(\varepsilon_y^R)$ $N_{a+1,y+1} = N_{a,y}\exp(-Z_{a,y}) \quad \forall a \in (1, \ldots, A-1)$ $N_{A,y} = N_{A-1,y-1}\frac{\exp(-Z_{A-1,y-1})}{1-\exp(-Z_{A,y-1})}$, where $\varepsilon_y^R \sim \text{Normal}(0, \sigma_R)$, ϕ_0 gives unfished spawning biomass per recruit, and ϕ_F gives spawning biomass per recruit at the initial F_y level
Mature biomass	S_y	$S_y = \sum_a N_{a,y}w_am_a$, also referred to as spawning biomass
Population biomass	B_y	$B_y = \sum_a N_{a,y}w_a$
Landed catch at age	$C_{a,y}$	$C_{a,y} = \frac{F_{a,y}}{Z_{a,y}}N_{a,y}[1-\exp(-Z_{a,y})]$
Landed age compositions	$p_{a,y}$	$p_{a,y} = \frac{C_{a,y}}{\sum_a C_{a,y}}$, proportional contribution of age class a in year y to the fishery
Data		
Observed landings	L_y	$L_y = \exp(\varepsilon_y^L)\sum_a C_{a,y}w_a$, where $\varepsilon_y^L \sim \text{Normal}(0, 0.05^2)$
Observed catch at age	$C'_{a,y}$	$\boldsymbol{C}'_y \sim \text{Multinomial}(n, \boldsymbol{p}_y)$, where $\boldsymbol{C}'_y$ and $\boldsymbol{p}_y$ denote vectors of age-specific quantities in year y
Observed abundance index	U_y	$U_y = \varepsilon_y^U q\sum_a N_{a,y}s_a$, where $\varepsilon_y^U \sim \text{Normal}(0, \sigma_I^2)$

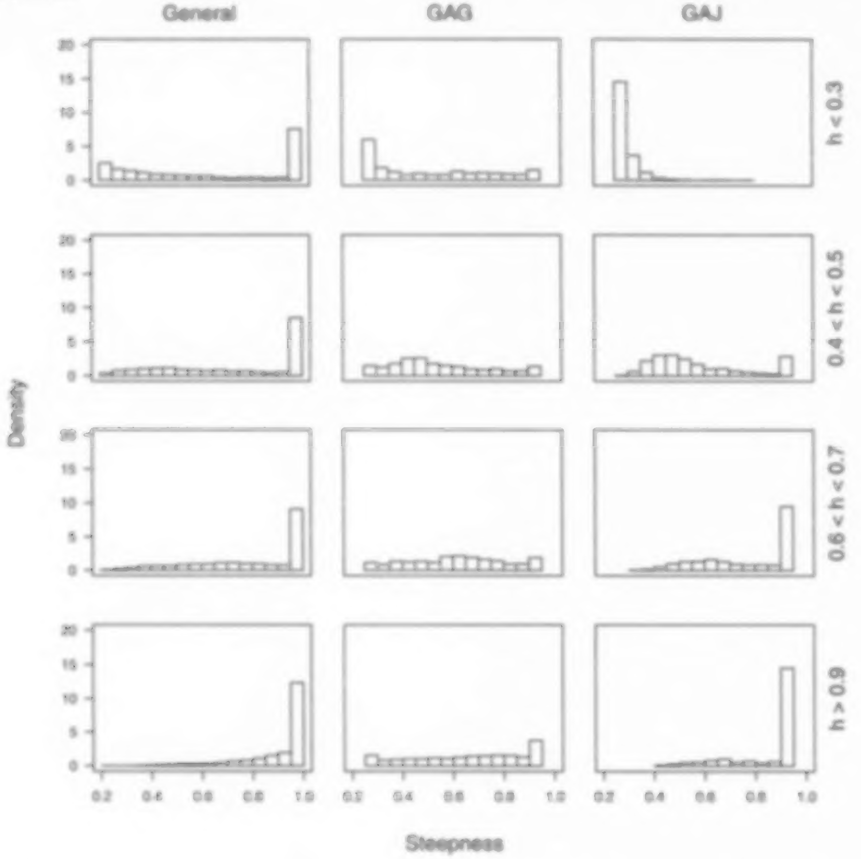
*These quantities estimated within SCA analyses.

Table 3. Description of log-likelihood components and penalties used in the assessment model for estimation. Predicted quantities are indicated with a tilde and follow the same functional form as in Table 2 but use estimated parameters instead of true values. Unlike the generating model, separate parameters are estimated for selectivity and maturity. A small constant (k = 0.00001) is added in several places to prevent numerical errors.

Quantity	Symbol	Description or definition
Multinomial age compositions	Λ_1	$\Lambda_1 = n\sum_y\left\{\sum_a (C'_{a,y}+k)\log[(p_{a,y}+k)]\right\}$
Lognormal landings	Λ_2	$\Lambda_2 = \sum_y \frac{\{\log[(L_y+k)/(\tilde{L}_y+k)]\}^2}{2(0.05)^2}$
Lognormal abundance index	Λ_3	$\Lambda_3 = \sum_y \frac{\{\log[(U_y+k)/(\tilde{U}_y+k)]\}^2}{2(\sigma_I^2)^2}$
Constraint on recruitment deviations	Λ_4	$\Lambda_4 = \sum_y R_y^2$, where R_y is the log of the recruitment deviation from the Beverton–Holt model in year y
Total likelihood	Λ	$\Lambda = \sum_{i=1}^{4}\Lambda_i$, objective function minimized by the assessment model

The primary motivation of these simulations was to understand which stock characteristics were the most important for precise and accurate estimation of steepness. As omniscient simulators, we were able to use known parameter values and stock trajectory statistics as candidate predictor variables for $\hat{h}$. To this end, we expressed eq. 2 as a function of simulation inputs and stock characteristics. Models were developed for logit(σ_h) and logit(π) that were written in terms of h, Y, M, σ_R, σ_I, n, F_{type}. In particular, we considered models ranging from an intercept-only model to one

Fig. 2. Histograms of estimated steepness, given different ranges of steepness used to generate data (ranges displayed on the right axis) for the general simulation study (left panels), gag grouper (*Mycteroperca microlepsis* (GAG); center panels), and greater amberjack (*Seriola dumerili* (GAJ); right panels).



with an additive effect of all predictor variables and an interaction between σ_R and F_{type} (where present, F_{type} was included as a factor variable). We excluded more interactions because they make parameter estimates difficult to interpret. A stepwise AIC model selection procedure with backward selection (Venables and Ripley 2002) was used to select submodels for logit(σ_h) and logit(π) that were of reasonable structure. Once a final model had been selected, we plotted predicted values over the range of each input parameter to examine the influence of a particular input on estimates of steepness.

Case studies

We considered two case studies to investigate estimates of steepness in real applications, reliability of these estimates, and the influence of the estimates on management benchmarks. For illustration, we selected greater amberjack (GAJ) and gag grouper (GAG), both located off the southeastern United States and recently assessed using SCA models (SEDAR 2006; 2007). For GAJ, steepness was estimated at 0.74 in the base model run, but several sensitivity runs estimated steepness at its upper bound, which was set to 0.95 in both assessments. For GAG, steepness was estimated at the upper bound in the base model run. In the original assessments, uncertainty around the benchmarks was quantified via a bootstrap procedure in which recruitment deviations were resampled a large number of times and parameters of the spawner–recruit curve were repeatedly re-estimated to generate distributions of MSY benchmarks. Many of the bootstrap iterations produced values of steepness at the upper bound, leading to bimodal distributions of benchmarks.

These two case studies highlight questions that arise in stock assessment whenever steepness is estimated. For instance, when steepness is estimated at the upper bound (as with GAG), does this provide some indication that the stock really is highly productive? Or, in such cases, are we better off adopting a proxy-based benchmark (see, e.g., Mace 1994; Williams and Shertzer 2003)? Can we be any more confident in our estimates when steepness is not estimated at the upper bound (as with GAJ)?

To answer these questions, we conducted simulations based on the exact number of years, sample sizes, gear types, and error structures used in each assessment. A complete description of model structure and data sources is described elsewhere (SEDAR 2006; 2007), but we provide a few relevant details here. Both assessment models had structure similar to that provided in Table 2, but with multiple sectors (commercial and recreational), multiple gears (e.g., handline, spears), and discard mortalities. Sampling of length and age compositions of landings began for most fisheries in the late 1970s or 1980s. Fishery-dependent indices of abundance were also available beginning in the 1970s. The GAJ assessment model spanned 1946–2006, and the GAG model spanned 1962–2004.

For each stock, we simulated data with process and observation error. All simulation parameters were fixed to estimates from the assessments, with the exception of steepness, which we sampled from a Uniform(0.21, 1.0) distribution. Note that both stock assessment models imposed boundaries on steepness at 0.2 and 0.95 so that the simulated and estimated ranges differed slightly for the case studies. The same lognormal probability distributions estimated in the assessments were used to generate spawner–recruit deviations. That is, the sampled value of steepness was used to determine the underlying spawner–recruit curve, and random lognormal recruitment deviations were generated using the value of σ_R estimated in the original stock assessment. Each simulated data set was then analyzed with the assessment model, and this process was repeated 10 000 times.

As with the general simulation study, estimates of steepness ($\hat{h}$) in the case studies were assumed to be related to true steepness (h) via a normal mixture model. For both case studies, we assumed that the probability of steepness being estimated at its upper bound was a linear function of steepness on the logit scale:

$$\text{logit}(\pi_i) = \beta_1 + \beta_2 h_i^{\text{true}}$$

However, in contrast to the general simulation study, preliminary investigation of the GAJ data suggested that steepness estimates not occurring at the upper bound were negatively biased and that the magnitude of bias depended on the true steepness value. As such, we based inference on eq. 2, but considered the model

$$\text{logit}\left(\frac{\mu_h - 0.2}{0.75}\right) = \beta_3 + \beta_4 h_i^{\text{true}} + \beta_5 (h_i^{\text{true}})^2$$

This transformation constrains the predicted mean steepness value to fall within the (0.2, 0.95) range imposed by the SCA analyses in the stock assessments and allows bias in estimates of steepness to be a nonlinear function of the true steepness value. We also assumed a linear relationship between the standard deviation of the continuous part of the mixture distribution and true steepness:

$$\text{logit}(\sigma_i) = \beta_6 + \beta_7 h_i^{\text{true}}$$

We applied Markov chain Monte Carlo (MCMC) to sample each stock's posterior steepness distribution, using data from the simulation experiments for calibration (see Appendix A for details). This distribution was then used to calculate a posterior distribution of F_{MSY}. We compared these distributions with those obtained from 1000 bootstrap samples, where estimated spawner–recruit deviations were resampled and steepness was re-estimated (externally) at each iteration. The latter approach was used to characterize uncertainty in the peer-reviewed stock assessments of GAJ and GAG. By conditioning on an F-weighted selectivity vector and on assumed growth, maturity, and natural mortality schedules, one could then solve for the corresponding distribution of F_{MSY} (SEDAR 2006).

Results

General simulation study

Overall, 56.5% of simulations had h^I estimated at its upper bound, and 36.9% had h^E estimated at its upper bound. MARE was also larger for internal estimates (0.227) than for external ones (0.193), suggesting that external estimates may be preferable (at least for the configuration used in this section). Subsequent simulation analyses thus use $\hat{h}^E$ as the dependent variable.

Inspection of histograms of $\hat{h}^E$ over different ranges of true steepness values suggested a bimodal distribution for estimated steepness (Fig. 2). The stepwise model selection procedure for π provided a high degree of support for all predictor variables (removing the least influential predictor resulted in an increase of 105 AIC points). However, predictive plots of the effect of each input variable clearly showed that some predictors were more important than others in determining whether steepness was estimated at its upper bound (Fig. 3). In particular, $\hat{\pi}$ increased substantially as the true value of steepness increased and as the coefficient of variation associated with the index of abundance increased. In contrast, the number of years of the study, natural mortality, age composition sample size, and recruitment variability appeared to be less important. The exploitation pattern (a proxy for biomass contrast) also appeared to be quite important, with two-way trips substantially decreasing (but not eliminating) the expected probability of having steepness estimated at its upper bound.

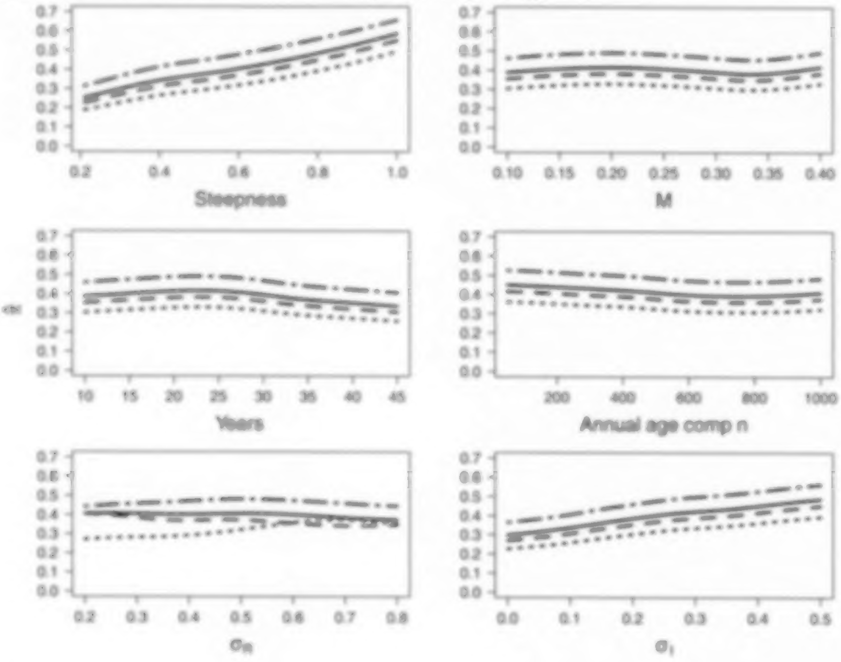
For the GLM analysis of factors affecting σ_h, the model with all predictor variables but n (the annual age composition sample size) had the lowest AIC score and was selected for effect comparisons. Here, the precision of $\hat{h}$ tended to increase whenever true steepness, natural mortality, or the number of years of the study increased, or when the index of abundance had smaller standard error (Fig. 4). Precision was the best for two-way trips and for high but constant levels of fishing mortality. One-way trips resulted in slightly less precision, with constant but low levels of fishing mortality providing substantially less precise estimates. The effect of recruitment variability on σ_h differed depending on exploitation profile: higher recruitment variability resulted in more precise estimates when exploitation was constant but less precise estimates when fishing mortality rates varied from year to year (as a function of either one- or two-way trips).

Case studies

Greater amberjack

Greater amberjack simulations resulted in estimates of steepness that were more precise than those estimated in the general simulation study. However, steepness was frequently estimated at the upper bound when true steepness was

Fig. 3. Predicted probability of steepness being estimated at 1.0 ($\hat{\pi}$) as a function of simulation inputs. Predictions are generated by varying x-axis values while fixing all other input variables to their mean. Input variables include the true value of steepness (steepness), natural mortality (M), the number of years for which data are available (years), the annual sample size for age composition data (n), the standard deviation of spawner–recruit errors (σ_R), and the assumed lognormal standard deviation for relative abundance data (σ_I). Solid lines give predictions for constant fishing mortality with $F/F_{MSY} = 0.5$ ($F_{type} = 1$); broken lines for constant F at $F/F_{MSY} = 2.0$ ($F_{type} = 2$); dotted lines for a two-way trip ($F_{type} = 3$); and dashed-dotted lines for strictly increasing F ($F_{type} = 4$).



greater than about 0.5 (Fig. 2). Unlike the general simulation study, estimates hit the upper bound more frequently when steepness was estimated externally than internally. Accordingly, MARE was lower when steepness was estimated internally (0.091) than when estimated externally (0.125). Also, when steepness was not estimated at the upper bound, it tended to be biased for certain values of true, underlying steepness. For instance, the mode of steepness estimates was positively biased at lower steepness values (Fig. 2).

Application of inverse prediction produced a posterior distribution of steepness that was noticeably smoother than a distribution generated by bootstrapping spawner–recruit residuals (Fig. 5). The bootstrap procedure resulted in a bimodal distribution of steepness estimates, with the dominant mode at the upper bound and the secondary mode near 0.55. The inverse prediction procedure resulted in a unimodal distribution, with the majority of estimates greater than 0.55. The posterior mean and median of the inverse prediction density were 0.66 and 0.64, respectively, and thus closer to that estimated by the SCA analysis. Comparisons of F_{MSY} for the two approaches mirrored steepness results, with the bootstrap procedure resulting in a bimodal distribution and the inverse prediction method resulting in a unimodal posterior distribution (Fig. 5).

Gag grouper

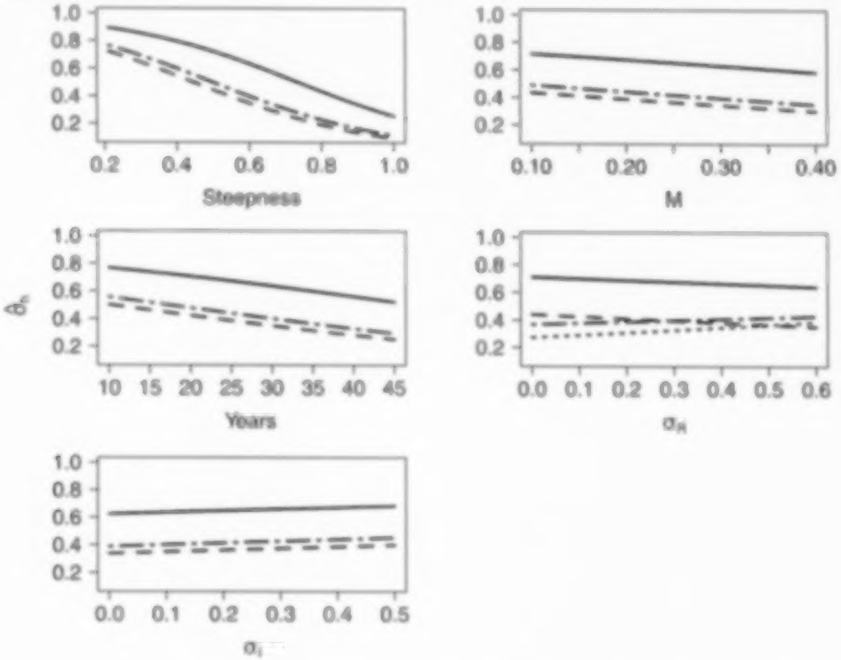
For gag grouper, simulations revealed little bias in estimates of steepness, although the estimator was imprecise (Fig. 2). Steepness was estimated at the upper bound less often than in general or GAJ simulations. In this case, the MARE for steepness was marginally lower when steepness was estimated externally (0.147) than internally (0.152).

Both inverse prediction and bootstrap procedures indicated that the stock was reasonably productive. However, inverse prediction once again outperformed the bootstrap approach, as it produced a posterior distribution of steepness that was smoother and with much less probability mass at the upper bound (Fig. 6). The posterior mean and median were 0.65 and 0.68, respectively, which were both lower than the SCA estimate of 0.95 from the GAG assessment. Paralleling the pattern in steepness, the bootstrap procedure resulted in substantial mass at $F_{MSY} = 0.24$, and the inverse prediction model admitted considerably more uncertainty (Fig. 6). For the inverse prediction approach, the posterior mean and median of F_{MSY} were both 0.14, suggesting that the value produced in the GAG assessment (0.24) may be too high.

Discussion

It is not uncommon in stock assessment for steepness to be estimated at its upper bound (e.g., GAG: SEDAR 2006). In this paper, we examined conditions in which steepness can and cannot be estimated reliably, as well as the factors that are important in discriminating between the two cases. In general, we found that the true steepness value and the

Fig. 4. Predicted standard deviation for estimated steepness as a function of simulation inputs for cases in which steepness was not estimated at its upper bound. Solid lines give predictions for constant fishing mortality with $F/F_{\mathrm{MSY}} = 0.5$ ($F_{\mathrm{type}} = 1$); broken lines for constant F at $F/F_{\mathrm{MSY}} = 2.0$ ($F_{\mathrm{type}} = 2$); dotted lines for a two-way trip ($F_{\mathrm{type}} = 3$); and dashed–dotted lines for strictly increasing F ($F_{\mathrm{type}} = 4$). The dotted line is indistinguishable from the broken line in most of the graphs. Simulation inputs include the true value of steepness (steepness), natural mortality (M), the number of years for which data are available (years), the standard deviation of spawner–recruit errors (σ_R), and the assumed lognormal standard deviation for relative abundance data (σ_I). The sample size of annual age compositions was not selected by AIC, so parameter effects are not presented here. Where not otherwise indicated, predictions are generated by evaluating the estimated GLM at the mean of other input variables.



exploitation history of the stock are important determinants of (*i*) whether steepness is estimated at its upper bound, and (*ii*) the precision of the steepness estimator. Two-way trips afforded the highest probability of estimating steepness precisely and accurately; in general, precision and accuracy increased as the number of years of data, quality of data (e.g., index CV), and natural mortality increased. Although the probability of estimating steepness at its upper bound increased as true steepness increased, it was still quite possible to arrive at such an estimate when productivity was low.

Our results differed somewhat from those of other studies that used SCA analysis to investigate the sensitivity of management benchmarks and model parameters (including h) to simulation inputs. For instance, Magnusson and Hilborn (2007) found that the reliability of estimates was much more dependent on having a good contrast in spawning stock biomass than on exploitation pattern (e.g., a one-way or two-way trip). In contrast, we found that two-way trips were quite important, improving both the accuracy and precision of estimates. However, the depletion levels attained in each study likely differ, and one-way trips tended to reach a lower depletion level than two-way trips in Magnusson and Hilborn's (2007) study.

Like Haltuch et al. (2008), we found that the length of the catch time series was an important predictor of the precision of steepness; however, it did not appear as important a determinant for h being estimated at its upper bound. Haltuch et al. (2008) found that the variance of recruitment deviations was also important, with larger recruitment variances leading to less accurate steepness estimates. Our results were more equivocal, indicating that this finding varies as a function of exploitation regime. If exploitation patterns produce good contrast in biomass, increasing recruitment variation tends to decrease our ability to estimate steepness reliably. In contrast, if exploitation rates are held constant, it is only through high recruitment variance that one can hope to get contrast in stock biomass; in this case, increasing spawner–recruit variance can actually improve our ability to estimate steepness. Another difference between our study and that of Haltuch et al. (2008) concerned the importance of sampling intensity. Like Haltuch et al. (2008), we found that the sample size of age compositions was relatively unimportant. However, unlike their study, we found index variability to be an important predictor of estimator performance. Although we were unable to pinpoint the reason for this difference, we suspect that it is linked to the depletion levels achieved during the two simulation studies. In our general simulation study, populations were initialized assuming a constant, historical level of fishing that predated monitoring. In contrast, the simulations of Haltuch et al. (2008) assumed that stocks were started at virgin levels and were reduced by 65%–90% by the end of the study.

Fig. 5. Distributions of steepness ($\hat{h}^{I}$) and F_{MSY} for greater amberjack (*Seriola dumerili*). These distributions were generated in two ways: through bootstrap resampling (left panels) of spawner–recruit residuals and by using inverse prediction (right panels) to estimate a posterior distribution. Vertical broken lines represent values from the SCA analysis.

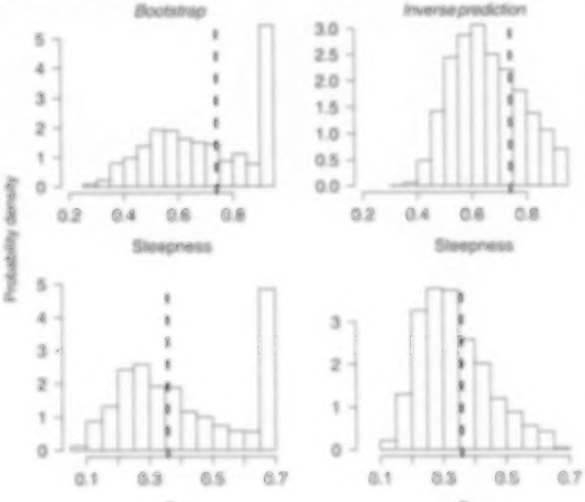
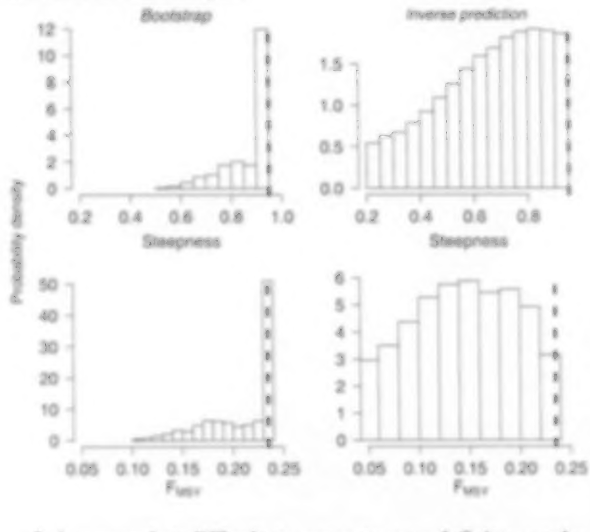


Fig. 6. Distributions of steepness ($\hat{h}^{I}$) and F_{MSY} for gag grouper (*Mycteroperca microlepsis*). These distributions were generated in two ways: through bootstrap resampling of spawner–recruit residuals (left panels) and by using inverse prediction (right panels) to estimate a posterior distribution. Vertical broken lines represent values from the SCA analysis.



It is somewhat difficult to come to any definite conclusions based on comparisons of previous work and the study in this paper, in part because of a lack of adequate controls. For instance, each study differed in simulation design, types of data considered, model formulation, and objective functions. However, speculation is not entirely idle in this case. Clearly, contrast in spawning biomass is important for estimating steepness; given equal contrast between stocks, our results suggest that two-way trips may result in better performance than one-way trips. Also, length of the catch time series appears to be a consistently important factor among studies, with longer time series leading to better performance of the steepness estimator. Similarly, age composition sample sizes do not appear to matter as much (although accuracy of age compositions may still be important). What is also clear from these studies is that although these factors can improve estimator performance, they do not guarantee that we will arrive at reliable estimates. For instance, we have shown here that simulations with good spawning biomass contrast and reasonable catch time series duration would still frequently result in steepness estimated at the boundary.

The general simulation study provided information on accuracy and precision of steepness in a relatively simple fishery (relatively short time series, a single index of abundance, a single gear, etc.). In contrast, case study simulations for GAJ and GAG were more detailed, mirroring the data sources and model structure used in the recent stock assessments. In these case studies, the accuracy and precision of steepness estimates increased dramatically, particularly for GAJ. In this case, it was rare that steepness was estimated at its upper bound when true steepness was less than 0.5. This finding suggests that estimates at the upper bound can actually be informative in some cases, at least for indicating which values of steepness are unlikely. When not estimated at its upper bound, steepness appears to be negatively biased when stocks are moderately to highly productive, a finding that agrees with Ianelli (2002), who found a similar pattern when examining the reliability of productivity estimates for ocean perch on the US west coast. Unfortunately, it appears necessary to perform fishery-specific simulations to examine bias and precision of the steepness estimator for individual stocks. The same holds true for examining whether internally or externally estimated values of steepness are more reliable.

We found inverse prediction methods to be extremely useful for characterizing posterior distributions of steepness that factored in species-specific simulation results. For GAJ, the posterior mean and median of true underlying steepness were almost identical to those obtained from the actual assessment and used for management, providing reinforcement that this steepness value was reasonable. For GAG, the inverse prediction approach suggested that the stock was likely highly productive, but that low productivity could not be discounted. Bayesian statisticians often prefer the posterior mean and median because they minimize squared and absolute error loss, respectively (Bernardo and Juárez 2003); for GAG, these values were 0.65 and 0.68, respectively. From this perspective, it appears that the estimate of 0.95 from the original assessment may be overly optimistic. As with steepness, we believe that the implied posterior distributions for F_{MSY} are more useful than those based on bootstrapping.

The inverse prediction approach is somewhat analogous to constructing a profile confidence interval for steepness. The latter approach is applicable when stock assessment models have been written strictly in terms of likelihood components (i.e., no ad hoc penalties, likelihood weightings, etc.). For profile confidence intervals to be interpretable, one often needs to estimate a variance inflation factor to account for model misspecification or lack of fit (Burnham and Anderson 2002), in addition to estimating variance components internally or via the EM algorithm (Cotter and Buckland 2004; Deriso et al. 2007). Nevertheless, when interpretable, the profile confidence interval is preferable to the inverse prediction approach because the other parameters in the model are re-estimated when conditioning on particular steepness values. For instance, when comparing likelihood values with steepness fixed to a low value, we might expect a higher estimate of virgin recruitment (R_0) and lower estimates of fishing mortality. In contrast, the inverse prediction approach described in this paper conditions on virgin recruitment and fishing mortality rates that are originally estimated by the SCA analysis. We thus believe that the inverse prediction approach as described will tend to underestimate uncertainty. A possible solution is to vary more simulation inputs (e.g., R_0 and a fishing mortality scalar, b) when conducting a multivariate calibration experiment (Brown 1982; Benton et al. 2003).

We echo the caveat provided by Yin and Sampson (2004) and others that the simulation framework used here is a drastic simplification of how data are "generated" and collected in the real world. Despite tailoring some simulations to specific data features of individual stocks (for GAJ and GAG), data were analyzed using the same probability model, natural mortality rate, and CVs used to generate the data. As a general rule, in the real world, we would expect concurrent model and parameter misspecification. In addition, factors such as ageing error and recruitment autocorrelation will also tend to obscure the spawner–recruit relationship. If anything, realized estimates of steepness in the real world should be accorded more uncertainty than suggested here.

In light of our results, we suggest that stock assessment analysts exercise extreme caution in interpreting steepness estimates at the upper bound. In many cases, this result gives little indication of the true productivity of a stock, and overestimates of steepness would lead to overly optimistic management advice, particularly with regard to recovery projections for depleted stocks. Analysts should be equally cautious of steepness estimates at the lower bound. Although this phenomenon was rare in our SCA model configurations, it has been observed in other studies (e.g., in management strategy evaluations of hake; M. Haltuch, National Marine Fisheries Service, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, WA 98112–2097, personal communication, 2009) and would likely lead to overly pessimistic management advice. When steepness is estimated at either bound, application of proxy-based benchmarks or inverse prediction approaches may prove particularly useful. Likewise, knowledge of life history may prove useful for setting likely ranges on steepness (e.g., Rose et al. 2001; Goodwin et al. 2006; Brooks et al. 2009).

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Appendix A. Details on MCMC sampling for steepness under inverse prediction

We assume that a simulation experiment is used to relate n estimates of steepness from an SCA analysis, $\mathbf{h}^{est} = \{h_1^{est}, h_2^{est}, \cdots, h_n^{est}\}$, to true values of steepness used to generate the data ($\mathbf{h}^{true} = \{h_1^{true}, h_2^{true}, \cdots, h_n^{true}\}$). The two are related through a function of the form $h_i^{est} = f(h_i^{true}, \boldsymbol{\theta}_i) + \varepsilon_i$, which is supplied by the user (for example, by specification of a linear model). Here $\boldsymbol{\theta}_i$ denotes a collection of unknown parameters, and ε_i is a random error term. For convenience, let this relationship be formalized through a joint probability density function given by

$$[\mathbf{h}^{est} | \mathbf{h}^{true}, \boldsymbol{\theta}]$$

where $[X|Y]$ denotes the conditional distribution of X given Y.

We suppose that the investigator uses an SCA analysis to provide an estimate of steepness, h_0^{est}, for a single stock of interest. Understanding that h_0^{est} could have arisen in any number of ways, we propose to estimate a posterior distribution of true steepness, h_0^{true}. Following Racine-Poon (1988), this can be done by sampling from

$$\text{(A1)} \qquad [h_0^{true}, \boldsymbol{\theta} | \mathbf{h}^{est}, \mathbf{h}^{true}, h_0^{est}] \propto \\ [h_0^{est} | h_0^{true}, \boldsymbol{\theta}][\mathbf{h}^{est} | \mathbf{h}^{true}, \boldsymbol{\theta}][\boldsymbol{\theta}][h_0^{true}]$$

Here, $[h_0^{true}]$ is a prior distribution of the true steepness of the unobserved stock, and $[\boldsymbol{\theta}]$ specifies a joint prior distribution of remaining unknown parameters (i.e., those describing functional relationships in the calibration experiment). For purposes of this study, we set $[h_0^{true}]$ to be uniformly distributed on $(0.2, 0.95)$ to correspond to the configuration of the assessment model (we note that this range is different from the (0.21, 1.0) range used in the general simulation study). If data were available, another possibility would be to use meta-analysis to specify an informative prior distribution (cf., Myers et al. 1999; Dorn 2002). An improper but diffuse prior was specified for $[\boldsymbol{\theta}]$ by setting it proportional to a fixed constant.

For both case studies, we assumed that true and estimated steepness were related by a normal mixture model (i.e., eq. 2). Define an indicator variable z_i for the ith simulation, which takes on the value of one if $h_i^{\text{obs}} = u$ and zero otherwise. The normal mixture model may then be rewritten as

$$\text{(A2)} \qquad [\mathbf{h}^{\text{est}}|\mathbf{h}^{\text{true}}, \boldsymbol{\theta}] = \prod_{i=1}^{n} \pi_i^{z_i}(1-\pi_i)^{1-z_i} \left[\frac{\mathcal{N}(h_i^{\text{est}}; \mu_i, \sigma_i)}{\int_l^u \mathcal{N}(h_i^{\text{est}}; \mu_i, \sigma_i) \mathrm{d}\widetilde{h}_i^{\text{est}}} \right]^{1-z_i}$$

where the upper (u) and lower (l) bounds of integration are set to the limits used in the SCA analysis. The model for observed steepness of the stock of interest (h_0^{obs}) is quite similar. In this case, define $z_0 = 1$ if $h_0^{\text{obs}} = u$ and $z_0 = 0$ otherwise. We then have

$$\text{(A3)} \qquad [h_0^{\text{est}}|h_0^{\text{true}}, \boldsymbol{\theta}] = \pi_0^{z_0}(1-\pi_0)^{1-z_0} \left[\frac{\mathcal{N}(h_0^{\text{est}}; \mu_0, \sigma_0)}{\int_l^u \mathcal{N}(h_0^{\text{est}}; \mu_0, \sigma_0) \mathrm{d}\widetilde{h}_0^{\text{est}}} \right]^{1-z_0}$$

In practice, there are two possible cases related to whether or not $z_0 = 1$. If $z_0 = 1$ (as was the case for GAG), $[h_0^{\text{est}}|h_0^{\text{true}}, \boldsymbol{\theta}] = \pi_0^{z_0}$. Thus, when $z_0 = 1$, knowledge of σ and μ does not provide information about h_0^{true} (that is, σ and μ are ancillary for h_0^{true} in a formal statistical sense). If h_0^{true} is the only parameter of interest, this implies that the bracketed quantity in eq. A2 may be omitted during analysis. In contrast, if $z_0 = 0$, inference must simultaneously be drawn on all parameters.

For GAJ, we specified eq. A1 in terms of eight parameters: $h_0^{\text{true}}, \beta_1, \beta_2, \ldots, \beta_7$. We assumed the following relationships:

$$\text{(A4)} \qquad \begin{aligned} \text{logit}(\pi_i) &= \beta_1 + \beta_2 h_i^{\text{true}} \\ \text{logit}(\mu_i) &= \beta_3 + \beta_4 h_i^{\text{true}} + \beta_5 (h_i^{\text{true}})^2 \\ \text{logit}(\sigma_i) &= \beta_6 + \beta_7 h_i^{\text{true}} \end{aligned}$$

Three parameters were estimated for the GAG analysis: h_0^{true}, β_1, and β_2. The definition for β_1 and β_2 was the same as for GAJ (i.e., as specified in eq. A4).

For each stock, parameters were sampled cyclicly using Metropolis–Hastings updates on their respective full conditional distributions (Gelman et al. 2004). We implemented a "random walk" chain (Givens and Hoeting 2005), whereby proposals (i.e., candidate parameter values) were generated as random normal variates with means centered at the parameter value at the previous iteration. Several sets of parameters were subjected to a high degree of correlation and were updated together in blocks. For instance, proposals for β_1 and β_2 were generated as multivariate normal random deviates with mean set to the previous iteration's values and covariance specified to generate a correlation of −0.99. This modification substantially improved mixing. The parameters β_3, β_4, and β_5 were also updated together, with covariances chosen to achieve the correlations $\rho_{\beta_3,\beta_4} = -0.9$, $\rho_{\beta_3,\beta_5} = 0.9$, and $\rho_{\beta_4,\beta_5} = -0.9$. This modification improved mixing slightly, but autocorrelation was still high. The remaining parameters were updated independently. Standard deviations for Metropolis–Hastings proposals were specified so as to result in a 30%–40% acceptance rate, as suggested by Gelman et al. (2004). Convergence was determined by examining trace plots of individual parameters, and by computing Gelman–Rubin statistics (Gelman et al. 2004).

For GAJ, the MCMC sampling procedure was run twice, resulting in two chains of length 100 000. Gelman–Rubin statistics indicated convergence of all parameters after about 40 000 iterations; thus the last halves of each chain were combined to yield a posterior sample of 100 000. For GAG, mixing was much better; in this case, two chains of length 10 000 were run, and Gelman–Rubin statistics indicated convergence after about 2000 iterations. The second halves of the two chains were combined to yield a posterior sample of 10 000 from which posterior moments were calculated. All analyses were performed in the statistical programming language R (R Development Core Team 2007).

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Tidal movements and residency of subyearling Chinook salmon (*Oncorhynchus tshawytscha*) in an Oregon salt marsh channel

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Abstract: A novel application of full-duplex passive integrated transponder (PIT) tag technology was used to investigate movements of individual subyearling Chinook salmon (*Oncorhynchus tshawytscha*; fork length ≥ 60 mm) into and out of tidally flooded salt marsh habitat in the Salmon River estuary, Oregon, USA. PIT interrogation was effective, with mean tag detection ≥ 92%. Salmon movement peaked late during both flood and ebb tide periods, indicating that salmon did not drift passively. Most movements were in the direction of tidal currents, but 20% of individuals entered the channel against the ebbing tide. Individuals occupied the intertidal channel for a median 4.9 h and as long as 8.9 h per tidal cycle, and few were detected moving when water depth was <0.4 m. Some individuals used the channel on multiple successive tidal cycles, and others entered intermittently over periods of up to 109 days. Using an individual-based approach, we characterized diversity of juvenile Chinook salmon behavior within a marsh channel, providing insight into the value of such habitats for conservation and restoration of salmon populations.

Résumé : Une utilisation inédite de la technologie des transpondeurs passifs intégrés à transmission bidirectionnelle simultanée (étiquettes PIT) a servi à étudier les déplacements de saumons chinook (*Oncorhynchus tshawytscha*; longueur à la fourche ≥ 60 mm) âgés de moins d'un an lors de leur entrée ou sortie d'un habitat de marais salant inondé par la marée dans l'estuaire de la rivière Salmon, Oregon, É.-U. Le système d'interrogation des PIT est efficace avec une détection moyenne de ≥ 92 % des étiquettes. Les déplacements des saumons atteignent un maximum à la fin des marées montantes et descendantes, ce qui indique que les saumons ne dérivent pas passivement. La plupart des déplacements se font dans le sens des courants de marée, mais 20 % des individus pénètrent dans le chenal contre la marée descendante. Les individus se retrouvent dans le chenal interdital pour une médiane de 4,9 h et jusqu'à 8,9 h pendant un cycle de marée et certains ont été observés se déplacer dans des eaux de < 0,4 m de profondeur. Quelques individus utilisent le chenal pendant plusieurs cycles successifs de marée et d'autres y pénètrent de façon intermittente pendant une période aussi longue que 109 jours. En utilisant une approche basée sur l'individu, nous décrivons la diversité des comportements de jeunes saumons chinook dans un chenal de marais, ce qui fournit une perspective sur la valeur de tels habitats pour la conservation et la restauration des populations de saumons.

[Traduit par la Rédaction]

Introduction

During their juvenile migration from freshwater to marine habitat, ocean-type Chinook salmon (*Oncorhynchus tshawytscha*) may rear for prolonged periods in subsidiary and blind channel networks that connect mainstem estuarine channels with peripheral wetlands (Congleton et al. 1981; Simenstad 1983; Healey 1991). Such channel networks are often intertidal, necessitating twice-daily evacuation of wetland areas and redistribution of nekton communities across hundreds of metres of habitat as channels flood and drain with the tide (Rozas 1995; Gibson 2003). Despite obligatory tidal emigrations, mark–recapture experiments indicate that individual Chinook salmon may return to particular wetland channels for days to months, moving into flooded channel networks during high tides and retreating to subtidal habitats during low tides (Congleton et al. 1981; Levy and Northcote 1982; Shreffler et al. 1990).

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The use of tidal channels as salmon rearing habitats has been the subject of multiple studies over the past several decades. Research has emphasized seasonal patterns of salmon abundance (Congleton et al. 1981; Levy and Northcote 1982), salmon feeding habits and prey resources (Shreffler et al. 1992; Gray et al. 2002), and restoration and recovery of habitat function in formerly degraded wetlands (Shreffler et al. 1990; Miller and Simenstad 1997; Gray et al. 2002). Recent work suggests that the presence or condition of estuarine marsh habitat may be linked to life history diversity and hence resilience of salmon populations (Bottom et al. 2005*a*, 2005*b*). Understanding the behavior of individual salmon within estuarine wetlands is necessary to evaluate habitat – life history associations and to predict changes to salmon populations that might result from wetland degradation or recovery (Simenstad and Cordell 2000). Yet patterns of salmon movement within and among tidal channels — including the timing and duration of tidal excursions into marsh habitats — remain poorly understood, particularly at fine temporal and spatial scales.

One impediment to studying salmon behavior in tidal marshes has been difficulty in tracking individuals with ocean-type life histories that are most likely to reside in marsh habitat. Because such fish (typically 40–100 mm fork length) are too small to monitor using active radio or acoustic telemetry, active telemetry studies of salmon in estuaries have used larger yearling migrants (e.g., Moser et al. 1991; Miller and Sadro 2003; Schreck et al. 2006) and (or) hatchery-reared fish (Semmens 2008.) The few studies that have described movement of small salmon in intertidal marsh channels have relied on physical recapture of marked fish using seines or traps (Levy and Northcote 1982; Shreffler et al. 1990). Repeated capture and handling may alter fish behavior, however, and such conventional methods are poorly suited to resolving movements that occur over the short time scale of tidal cycles.

We sought to address this problem by using full-duplex (FDX) passive integrated transponder (PIT) tag technology (Prentice et al. 1990*a*). PIT tags are common tools in freshwater fish research, where detection systems incorporating stationary, in situ PIT antennas have allowed passive monitoring of fish movements at multiple scales in natural and simulated stream habitats (reviewed by Zydlewski et al. 2006). High-salinity water has limited application of PIT detection technology in marine and estuarine habitats by reducing the distance over which PIT tags can be detected (e.g., McCormick and Smith 2004). Adams et al. (2006) and Meynecke et al. (2008), however, both reported using half-duplex (HDX) PIT tag detection systems for monitoring the movement of fish in salinities ranging from 2 to 28 PSU. Both investigators used HDX PIT tags measuring 23.1 mm long × 3.4 mm in diameter and weighing 0.6 g in air. Such tags (the smallest HDX tags commercially available) are too large and heavy for use with subyearling salmon, but at the time of our study, FDX tags measuring 12.5 mm long × 2.07 mm in diameter and weighing 0.102 g in air were available. Improvements in the read range of FDX tags and improved performance and capability of the transceiver system (e.g., electromagnetic interference filtering, operation of multiple antennas from a single transceiver, and antenna design) recently have increased the feasibility of using FDX PIT tag technology with small fish in brackish water habitats.

We operated a stationary FDX PIT tag detector within a blind, tidal salt marsh channel of the Salmon River estuary, Oregon, for several months during the summers of 2004 and 2005, coincident with a conventional mark–recapture experiment in the estuary using FDX PIT tags. Our objectives were to assess the utility of this approach for monitoring movement of small salmon in shallow estuarine habitats, investigate the timing and duration of intertidal channel use by individual age-0 Chinook salmon, identify environmental limits (e.g., temperature, depth) on channel occupancy, and test whether patterns of tidal movement varied among individuals of different sizes or tagging location.

Materials and methods

Study area

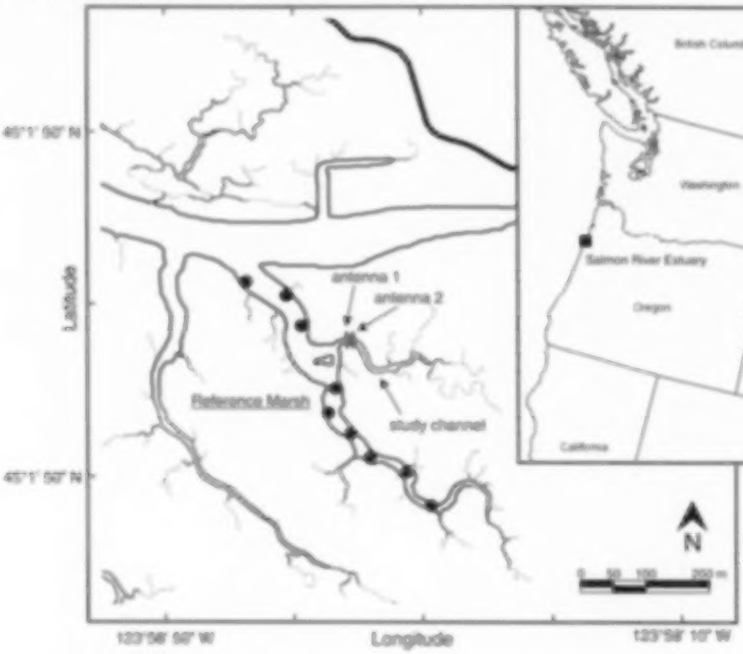
The Salmon River drains a catchment of 194 km^2 in the Oregon, USA, coastal mountains and flows into the Pacific Ocean at Cascade Head (45°01′N, 123°58′W; Fig. 1). The lowest 6.5 river kilometres (rkm) form a tidally influenced estuary, and the area of the estuary between rkm 2.0 and rkm 4.5 includes over 200 ha of salt marsh intersected by tidal marsh channels. Subyearling Chinook salmon are the dominant salmonids in the estuary, typically occurring in marsh channel habitats from March through October, with peak abundance in late spring or early summer. The estuary is the site of a long-term study of salmon rearing in tidal marsh channels (Gray et al. 2002; Bottom et al. 2005*a*).

Our study channel was a blind, secondary channel within a dendritic network of intertidal channels that joins the mainstem Salmon River at rkm 3.2 (Fig. 1). This larger channel network intersects an 80 ha, mature high salt marsh (Jefferson 1974) previously described as the Salmon River "reference marsh" or "control marsh" (Gray et al. 2002). Surface area of the study channel was approximately 2005 m^2, comprising 9.5% of the entire reference channel network. Width of the study channel at the detector site was 8 m, and water depth varied from ~1.5 m at high tide to <0.1 m on most summertime low tides. During both 2004 and 2005, high tide surface water salinity in the study channel varied from less than 5 PSU in June to 28–30 PSU in August and September. The water column was often stratified by salinity in late summer.

Fish tagging

During high tides between 18 May and 6 July 2004, juvenile Chinook salmon were captured by beach seining, PIT tagged, and released at nine sites within the primary channel of the reference marsh (N = 671) (Fig. 1). Tagging followed the techniques of Prentice et al. (1990*a*) using 12.50 mm long × 2.07 mm FDX PIT tags (model TX1411ST; Digital Angel Corp., St. Paul, Minnesota; weight 0.102 g in air). Prior to tagging, fish were anesthetized in a bath of ambient water from the marsh channel containing ≤50 mg·L^{-1} tricaine methanesulfonate (MS-222). Tags were inserted into the body cavity using a 12-gauge syringe, and tagged fish were allowed to recover from anesthesia in an aerated water bath before their release near the site of capture. Using the same methods, an additional 572 Chinook salmon were

Fig. 1. Map of study site in Salmon River estuary (Oregon, USA). Solid circles indicate locations where Chinook salmon (*Oncorhynchus tshawytscha*) were PIT tagged in the reference marsh in 2004 and 2005.



tagged and released at the same reference marsh sites between 28 June and 1 August 2005. Water temperature and salinity during tagging ranged from 9 °C to 19 °C and from 3 PSU to 18 PSU, respectively.

All tagged Chinook salmon were naturally produced (i.e., not spawned in a hatchery) and ranged from 60 to 116 mm fork length at the time of tagging. Previous studies have shown that PIT tagging has a minimal effect on survival, growth, and performance of salmonids of this size and larger (Prentice et al. 1990*b*; Ombredane et al. 1998; Newby et al. 2007). Tag weight ranged from 0.8% to 5.4% of body weight (median 3%). Brown et al. (2006) detected no effect on growth or swimming speed of juvenile Chinook salmon implanted with transmitters between 3.1% and 10.7% of body weight.

Detection equipment and data collection

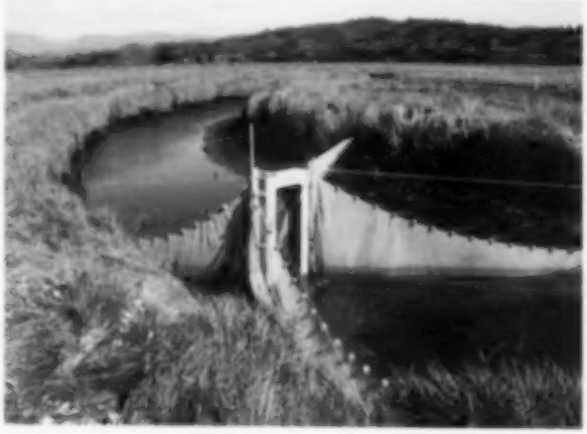
In 2004, the PIT tag detection system consisted of a single 24V FS-1001A transceiver (Digital Angel Corp.) powered by four 12V batteries and connected to one antenna anchored in the tidal channel. A Palm M130 handheld computer (Palm Inc., Sunnyvale, California) connected to the transceiver's serial port recorded time, date, and tag code information for each tag detection and periodically logged the transceiver's current, phase, and noise level using the program FS1001 v1.1 (Oregon RFID, Portland, Oregon).

In 2005, we replaced the FS-1001A transceiver with a newer model FS-1001M transceiver (Digital Angel Corp.). The FS-1001M was capable of multiplexing — operating up to six antennas by sequentially switching power to each antenna several times per second. To the new multiplexing transceiver, we attached the same antenna used in 2004 (antenna 1) and an additional antenna (antenna 2) anchored approximately 20 m farther into the study channel (Fig. 1). The two antennas enabled direction of fish movement to be determined via the time and date recorded with each tag detection. The FS-1001M transceiver was capable of tuning the antenna fields dynamically (automatic tuning) to maximize tag read distance. This feature resulted in improved tag detection efficiency across tidally and seasonally variable water depth and salinity. During 2005, tag detection and transceiver diagnostic data were logged with a Dell Axim handheld computer (Dell Inc., Round Rock, Texas) using the program MiniMon v1.4 (Pacific States Marine Fisheries Commission).

During both years, the transceiver, batteries, and data logger were contained within a 1 m × 1 m × 0.5 m stainless steel box anchored on the marsh surface above maximum high tide elevation. Batteries were exchanged weekly and recharged onshore.

Both antennas originally were designed and constructed by Digital Angel Corp. to detect PIT-tagged adult fish passage at McNary Dam on the Columbia River (Muir et al. 2001). Each antenna consisted of a continuous length of 14 AWG Teflon coated wire wrapped 13 times into a 170 cm × 64 cm rectangular coil. A "shield" of 24 cm wide, 6 mm thick, sheet aluminum channel surrounded the exterior of the wire coil with approximately 10 cm of open space between the shield and the coil on all sides. Both the antenna coil and aluminum shield were housed within an air-filled watertight housing of 18 cm × 27 cm fiberglass (antenna 1) or welded plastic (antenna 2) box channel. The inside dimension of these rectangular housings (i.e., the area that fish passed through to be detected) measured 50 cm × 157 cm (0.78 m^2) on each antenna. Laboratory testing using

Fig. 2. Antenna 1 installed in study channel at a moderately low tide.



FS-1001 transceivers under RF noise conditions similar to the Salmon River study site indicated that these antennas detected model TX1411ST PIT tags when the transceiver current was as low as 1.9 A.

We attached each antenna to two wooden posts driven into the marsh channel substrate, with the long axes of the antennas oriented vertically and located so that the antenna openings were centered on the thalweg of the tidal channel. Nets (0.5 cm mesh) extended from the wooden posts to the shoreline both above and below the antennas to direct fish through the antenna openings (Fig. 2).

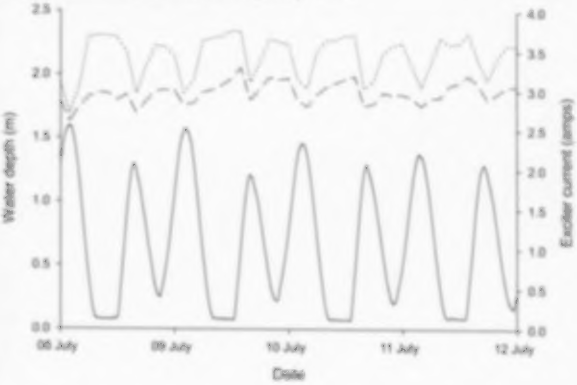
In 2004, we operated the detector system from 15 June to 10 July and from 18 August to 21 October. We removed the nets in mid-July to avoid interfering with an ongoing long-term fish trapping study in the study channel. In 2005, the detector operated continuously from 28 June through 12 September, except for five days when the transceiver shut down due to low battery power.

During 2004, we monitored water temperature at the detector site with a HOBO temperature logger (model H8; Onset Computer Corp., Bourne, Massachusetts). In 2005, we monitored both water temperature and depth with a data logger positioned on the channel bottom between the two antennas (HOBO model U-20-001-01; Onset Computer Corp.). Salinity at the water surface and within the water column was recorded at least twice a week during both years using a handheld refractometer or electronic salinity meter (YSI, Inc.).

Detection efficiency

Periodically during high tides, we verified the ability of the detection system to recognize PIT tags by passing test tags taped to a stick through the center of each antenna. Twice in 2004 and five times in 2005, we also released groups of tagged salmon into the study channel above the detector system during high tides to test the efficiency with which the nets and antennas combined to detect tagged fish moving out of the study channel. Twenty-seven such "efficiency fish" were released in 2004 and 76 were released in 2005.

Fig. 3. Exciter current of antenna 1 (dotted line) and antenna 2 (broken line) across a typical series of tides, indicated by water depth (continuous line), during July 2005.



Efficiency testing was based on the assumption that all fish released above the antennas would leave the channel as it dewatered on the ebbing tide. Accordingly, we defined detection efficiency as the percentage of each group of efficiency fish detected on the first ebbing tide after release. Because efficiency fish released in 2005 must have passed antenna 2 to reach antenna 1, the efficiency with which antenna 2 detected fish exiting the study channel was defined as the percentage of efficiency fish detected on antenna 1 that were also detected on antenna 2. Similarly, fish that were tagged and released outside of the study channel (i.e., in the adjacent, higher-order reference channel; Fig. 1), subsequently moved into the channel volitionally, and were detected on antenna 2 provided a means to evaluate the efficiency with which antenna 1 detected fish entering the study channel. We could not independently assess the efficiency with which antenna 2 detected incoming fish, but we have no reason to believe that it would differ substantially from the outgoing efficiency.

Results

Performance of detection equipment

Brackish water flooding increased the electrical load on antennas and caused transceiver current to vary tidally, decreasing as the amount of water in the channel increased (Fig. 3). Transceiver current ranged from 2.4 to 6.2 A but never dropped below the 1.9 A threshold of tag detection, even when salinity was highest during late summer. Water depth accounted for a large proportion of the observed variation in current for each antenna (linear regression of transceiver current on water depth in 2005, $r^2 = 0.93$ for antenna 1, $r^2 = 0.70$ for antenna 2; $p < 0.0001$). Test tags passed manually through the center of the antenna fields during high tides confirmed that both antennas maintained sufficient electromagnetic field strength to detect tags throughout the tidal cycle at surface water salinity as high as 29 PSU.

Detection rate of tagged efficiency fish exiting the channel ranged from 69% to 100% (mean 92%; Table 1). During the two tests with efficiencies lower than 90%, efficiency fish had been distributed particularly high in the study chan-

Table 1. Detection efficiency of tagged Chinook salmon (*Oncorhynchus tshawytscha*) released into study channel at high tide (*i.e.*, efficiency fish).

		Number detected				Efficiency (%)		
Date	Number released	Both antennas	Antenna 1 only	Antenna 2 only	Not detected	Antenna 1	Antenna 2	Overall
18 August 2004	13	na	9	na	4	69	na	69
16 September 2004	14	na	14	na		100	na	100
30 June 2005	31	13	8	5	5	68	62	84
7 July 2005	10	10				100	100	100
13 July 2005	12	11	1			100	92	100
20 July 2005	13	12			1	92	100	92
1 August 2005	10	9	1			100	90	100
Mean						90	89	92

Table 2. Summary of Chinook salmon (*Oncorhynchus tshawytscha*) tagged and released in Salmon River estuary outside of study channel and subsequently detected by PIT antenna during 2004 and 2005.

					Time-at-large	
Tagging dates	Individuals tagged	Total detections	Individuals detected	% detected	Median (days)	Maximum (days)
18 May – 6 July 2004	697	493	123	18	16	128
28 June – 1 August 2005	572	369	75	15	9.5	48

nel system, which may have reduced the likelihood of exiting the channel on the ebbing tide. Also, the large number of efficiency fish released on 30 June 2005 may have resulted in "tag collision", a condition when two or more tags are in the detector field at the same time (Connolly et al. 2008), reducing detection efficiency. Antenna 1 also detected 89 of 92 individuals (97%) that were released outside of the study channel, subsequently entered the channel, and were detected on antenna 2. Following efficiency tests, most efficiency fish (90%) returned to the study channel on subsequent tides (up to 32 days following release), and the system recorded a total of 91 and 224 detections of these fish in 2004 and 2005, respectively. Detections of efficiency fish were not used for other analyses of salmon movements or residence times.

Detection of tagged salmon

In 2004, the PIT detector recorded 493 detections of 123 unique individual tagged salmon that moved into the study channel volitionally after being released elsewhere in the estuary. These individuals included 18% of Chinook salmon tagged and released in the reference marsh (Table 2). In 2005, the system recorded 369 detections of 75 unique fish, 15% of Chinook salmon tagged in the reference marsh but outside the study channel.

During both years, the body size of salmon detected in the study channel was representative of the tagged population. Likelihood of detection and duration over which individuals used the study channel did not appear related to body size when tagged. Likelihood of detection in the study channel was greater for fish captured and tagged at sites above the confluence with the study channel than for those tagged and released below the confluence (Fig. 4; *G* test with Williams correction used to compare fish tagged above and below the channel, $p = 0.0014$ in 2004 and $p = 0.0025$ in 2005).

Tagged fish were detected between 5 h before and 6 h after high slack tides. Frequency distributions of all detections for both years indicate that peak movement of tagged salmon through the antennas occurred roughly 1 to 2 h before and 3 to 4 h after high slack tides (Fig. 5*a*). Tagged salmon were detected during both daytime and nighttime tides. Of 862 total detections during both years, roughly half (449 or 52%) occurred between 1 h before sunset and 1 h after sunrise.

Among all detections at both antennas during 2005, median water depth was significantly shallower when fish were detected during ebb tides than during flood tides (median depth 0.75 m vs. 0.95 m, Wilcoxon rank sum test $p = 0.0012$). Results from 2004, when we did not measure water depth directly, were qualitatively similar. Only 21 (6%) of the total detections in 2005 occurred when water depth was 0.4 m or less, despite the fact that shallow depths made up the greatest proportion of depths recorded at the antenna site (Fig. 6). Of these 21 detections, 17 (81%) occurred during low light conditions between 1 h before sunset and 1 h after sunrise.

Median water temperature at the bottom of the channel when fish were detected was 14.9 °C (interquartile range 13.7 to 16.0 °C) in 2004 and 16.4 °C in 2005 (interquartile range 15.0 to 17.4 °C). High tide water temperature did not appear to limit salmon from using the channel. The warmest temperatures (exceeding 25 °C) occurred during low tides when water was shallow and tagged fish were not moving in the study channel (see Fig. S1 of the supplementary data).[4]

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[4] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5349. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Fig. 4. Proportion of individuals detected in the study channel after initial capture at nine tagging sites within the reference marsh channel network. Data are grouped by tagging site and arranged by distance of tagging site from the mainstem Salmon River. Vertical dotted line indicates location of study channel confluence. Sample sizes of fish tagged are indicated for each site.

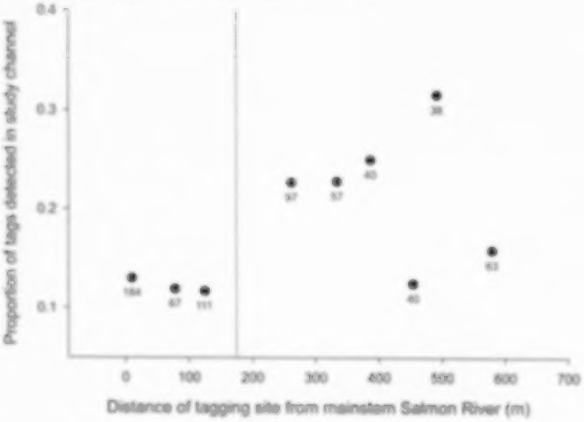


Fig. 5. (*a*) Frequency of all PIT tag detections at antenna 1, and (*b*) frequency of fish movements into (solid bars) and out of (hatched bars) the study channel in 2005, relative to the tidal cycle. Vertical dotted lines indicate timing of high slack tide.

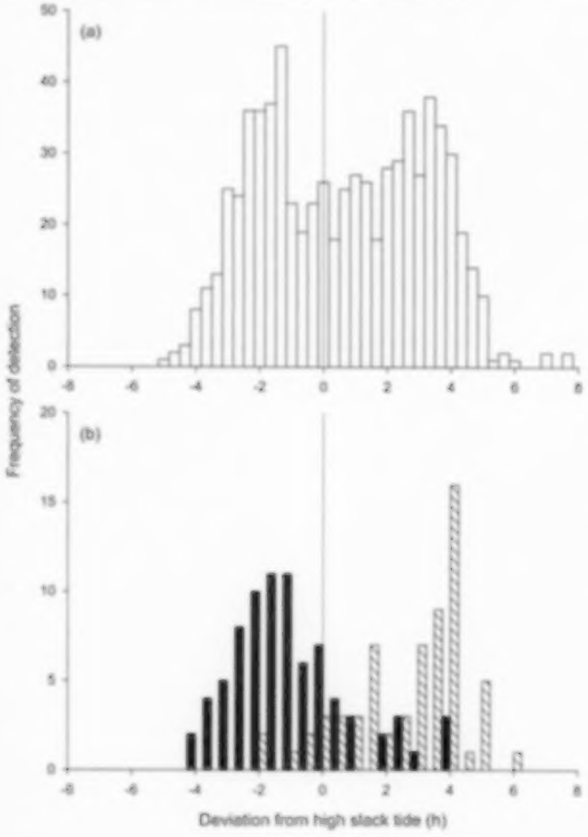
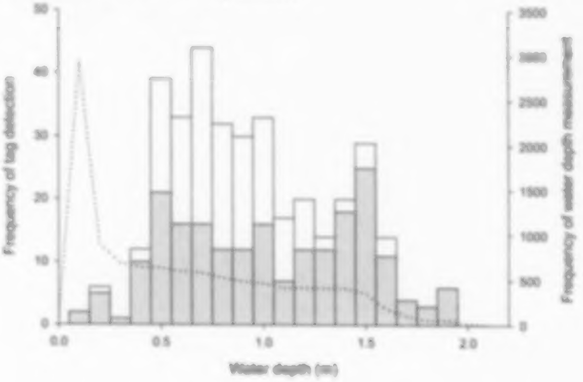


Fig. 6. Frequency of PIT tag detections by water depth at the detector site in 2005. Shaded portions of bars represent detections that occurred during low light conditions. Dotted line indicates frequency of depth records during the period of time that fish were detected (29 June – 13 September).



Patterns of movement and residence time of individual salmon

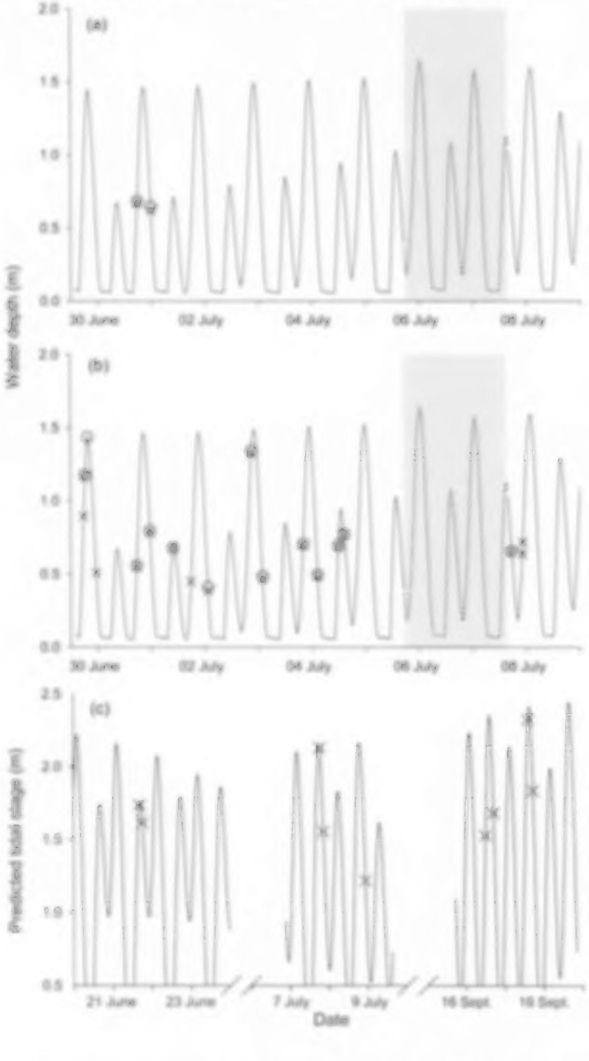
Most individuals recorded by the PIT tag detector were detected on few occasions — a median of two detections per fish — but several individuals were detected on multiple occasions, including single individuals that were detected up to 44 times in 2004 and 33 times in 2005. Most fish (~65% of those detected) occurred in the study channel during one or two tidal cycles within a few days of tagging and then did not enter the channel again (Fig. 7*a*). Of the remaining fish, some individuals demonstrated fidelity to the study channel, entering and leaving the channel over multiple successive tidal cycles (as often as 22 tide cycles in a 24-day period) (Fig. 7*b*). Others were detected on two or more tide cycles separated by several days or weeks without detection, indicating they used the study channel only sporadically during a prolonged period of estuarine residence (Fig. 7*c*).

The maximum times-at-large between initial tagging in the reference marsh and final detection in the study channel were 128 days in 2004 and 48 days in 2005. Median times-at-large were 16 days and 9.5 days after tagging in 2004 and 2005, respectively (Table 2).

Using individual detection data from both antennas in 2005, we defined 80 clear entrance movements (individuals detected successively on antenna 1 then antenna 2) and 65 clear exit movements (detected on antenna 2 then antenna 1; Fig. 5*b*). Most fish moved in the direction of tidal currents: 80% entered during flooding tides and 92% exited on the ebb, 20% entered on the ebb, and 8% exited on the flood. The timing of entrance and exit movements was consistent with periods of peak tag detection before and after high slack tide.

On 57 occasions, individual fish exhibited clear entrance and exit movements during single tidal cycles. Based on these 57 observations (from 36 unique individual fish), the median individual residence time within the channel per tidal cycle was 4.9 h (range 0.37 h to 8.9 h). Residence time showed a weak but significant positive association with tidal magnitude (linear regression of residence time on depth in the channel measured at high slack tide, $r^2 = 0.16$, $p = 0.002$). The longest

Fig. 7. Complete detection histories of three individual salmon demonstrate diverse patterns of marsh residence: (*a*) a typical fish that used the channel on only one tidal cycle after being tagged on 28 June 2005 (85 mm and 7.3 g when tagged); (*b*) an individual that was tagged on 28 June 2005 (86 mm and 7.3 g) but showed fidelity to the study channel over several tidal cycles; (*c*) an example of occasional use of the channel by one individual over several months during 2004 (tagged 7 June 2005 at 72 mm and 4.3 g). Detection on antenna 1 is indicated by ×; detection on antenna 2 is indicated by ○ (no antenna 2 in 2004). Continuous lines represent the tidal cycle as water depth at the study site (2005) or predicted tidal stage (2004). (The shaded areas in (*a*) and (*b*) indicate a period 5–7 July 2005 when the PIT detector was not operating.)



observed residence times were associated with particularly high tides that occurred at night. On a given tidal cycle, individual fish tended to exit the channel when the water depth was equivalent to or shallower than the depth when they entered. The mean difference in depth between entrance and exit was 20 cm (standard error 5 cm), and water depth at exit was significantly shallower than depth at entrance (one-sided *t* test, $t_{[56]} = -3.54$, $p = 0.0004$).

Discussion

Development of FDX PIT tag technology has allowed quantification of individual small fish movement within intertidal salt marsh habitat on the temporal scale of individual tidal cycles. By monitoring movements of PIT-tagged subyearling Chinook salmon into and out of a tidal wetland channel, we found evidence that (*i*) salmon used marsh channel habitat over a broad range of tidal conditions when water depth was greater than 0.4 m; (*ii*) individual salmon remained in the intertidal channel for a median 4.9 h and as long as 8.9 h per tidal cycle; (*iii*) the timing of salmon movement into and out of intertidal habitat was not centered on high slack tide, and fish at times swam against tidal currents; (*iv*) salmon exhibited some fidelity to a specific intertidal channel within the estuarine marsh landscape; and (*v*) individuals within the population exhibited diverse patterns of residence in the study channel, ranging from hours to months, including periods of continuous and infrequent habitat use.

Although many studies have documented estuary-wide migrations, residence times, and habitat use by juvenile salmonids (e.g., Healey 1980; Kjelson et al. 1982; Miller and Sadro 2003), few have examined the fine-scale movement of individual salmon into and out of shallow marsh habitats as channels drain and fill with each tide (Levy and Northcote 1982). New micro-acoustic tags have been used to study estuarine migration behaviors and survival rates of juvenile salmon (Williams 2008), as well as the fine-scale movements of individuals placed within estuarine habitat enclosures (Semmens 2008). However, such tags are too large for applications involving the small (<90 mm) size classes of salmon that typically reside in wetlands and other shallow estuarine habitats (Levy and Northcote 1982; Simenstad et al. 1982; Bottom et al. 2005*b*).

Although juvenile salmon usually entered and departed the Salmon River marsh channel in the direction of tidal currents, the asymmetry of salmon movement about high slack water and the high proportion of movements that occurred against ebbing tides indicate that Chinook salmon do not drift passively with the current, but rather enter and actively remain in intertidal habitat until late in the tidal cycle. Peak salmon movement occurred during mid- to late flood tides (i.e., 1–2 h before high slack tide) and late during ebb tides (i.e., 3–4 h after high slack tide). Furthermore, 20% of individuals entered the channel against the ebbing tide, 8% exited against the flooding tide, and fish exited the channel at water depths that were, on average, 20 cm shallower than when they entered. Using trap nets set across marsh channels at high tides, Levy and Northcote (1982) similarly reported that most Chinook salmon and chum salmon (*Oncorhynchus keta*) fry in the Fraser River estuary left intertidal marsh channels during late ebb-tide periods, whereas pink salmon (*Oncorhynchus gorbuscha*) emigrated during early and middle stages of ebbing tides. Pink salmon occur in estuaries at much smaller sizes than Chinook salmon and thus may be less able to maintain position against tidal currents than larger species. Our results for Chinook salmon differ from patterns observed for nekton in an intertidal creek in South Carolina where most taxa departed the channel at the same depth as they entered (Bretsch and Allen 2006).

Behavioral studies have demonstrated that habitat choices by juvenile salmon involve species-specific tradeoffs between optimal foraging opportunities and predation risks (Magnhagen 1988; Abrahams and Healey 1993) or physiological stresses (Webster et al. 2007). Salt marsh habitats provide productive feeding habitats for juvenile salmon (Levy and Northcote 1982; Simenstad et al. 1982; Shreffler et al. 1992) and have been described as potential predator refugia (Shreffler et al. 1992), but the relative benefits and costs for salmon occupying shallow intertidal marsh channels rarely have been measured (Craig and Crowder 2000). The patterns of salmon residence in the Salmon River study channel may serve to maximize foraging success while limiting predation risks and the bioenergetic costs of increasing water temperatures (Gray 2005). Tagged fish avoided shallow water where avian predation, potential for stranding, and elevated water temperatures may pose increased risks of mortality during receding tides. Occasions when fish were detected in shallow water were usually periods of low light, when risk from visual predators was minimized. Chinook salmon feed actively in Salmon River marsh channels on invertebrate taxa produced within the marsh (Gray et al. 2002). By remaining within the channel as the tide ebbs, individuals may maximize encounters with drifting invertebrate prey exported from the marsh channel network and concentrated during receding tides.

Despite having to vacate tidal channels with each ebb tide, our results indicate that individual salmon may exhibit fidelity at the scale of secondary marsh channels within an intertidal marsh–channel network. For example, some individuals used the study channel repeatedly on successive tides. Moreover, fish tagged in the reference marsh network above the confluence with the study channel were more likely to be detected than those tagged below the confluence. Presumably, this was because fish in the upper portion of the marsh system again penetrated high into the channel network on subsequent tides. Although many individuals that were detected multiple times entered the study channel only sporadically, it is unclear whether such fish were regularly using another, unmonitored area within the reference network. Because the entire population of the intertidal channel network is concentrated within a limited number of subtidal refuge habitats in the mainstem estuary during low tide, some redistribution of individuals within the network likely occurs with each tidal cycle. Consequently, site fidelity may be weaker at finer spatial scales within intertidal channel networks than at coarser scales among networks.

Through continuous monitoring of fish entering and exiting the study channel, our PIT tag methodology discerned considerable variation in the wetland rearing behaviors of individual salmon that may not be apparent from conventional survey methods. For example, some individuals revisited the same small wetland channel intermittently for periods of weeks or months. Conventional methods that use fixed nets to sample periodically (e.g., daily, weekly, or monthly) therefore may greatly underestimate wetland residency and cannot measure variation in the frequencies of tidal excursions among individuals entering and leaving local habitats. The small but noteworthy proportion of tagged Chinook salmon that we observed entering the study channel during ebbing tides would have been excluded from the channel — and thus not included in abundance estimates — by traditional sampling methods that place trap nets across the mouths of tidal channels at high slack tide (e.g., Levy and Northcote 1982; Shreffler et al. 1990; Miller and Simenstad 1997). Such methods also may not detect residence patterns accurately if channels do not drain completely during neap low tides.

Most studies of marsh habitat use by estuarine nekton have sampled during daylight hours (reviewed by Rountree and Able 1993), which may greatly underestimate marsh channel use by animals that are more active or abundant at night (Rountree and Able 2007). Although we did not observe a substantial difference in the number of tagged salmon detected between night and day, the longest tidal residence times of individuals within the marsh occurred during nighttime tides. Passive, in situ tag monitoring did not require additional labor or expense to sample daytime and nighttime tides equally.

Our approach was limited somewhat by the dimensions of the PIT tag antennas available for this research, which required that we artificially narrow the study channel with nets. Improved, unshielded FDX PIT tag antennas measuring approximately 3 m × 0.5 m — about twice the detection area of the antenna used in the present study — since have been tested successfully in salinities of up to 28 PSU (E. Prentice, unpublished). Moreover, the small, shielded antennas that we used likely could detect smaller, 8 mm long FDX PIT tags that are now commercially available, potentially allowing monitoring of even smaller fish. These improvements and the ability to link multiple antennas to a single multiplexing transceiver offer considerable flexibility to span larger channels and to continually monitor the residency and movements of small fish in estuarine habitats.

Human development of coastal areas has altered many estuaries used by salmon (Boulé and Bierly 1987), and in some Oregon estuaries, as much as 80% of former tidal wetland area has become inaccessible to migratory fish as a result of diking, filling, and the installation of tide gates (Good 2000). Wetland restoration projects often attempt to recreate habitats that function equivalently to natural reference sites (Miller and Simenstad 1997; Gray et al. 2002), and fish behaviors, including residence times and movement patterns, have been proposed as important measures of restoration success (Simenstad and Cordell 2000). The fact that most tagged salmon occupied our intertidal study channel only when water reached a minimal depth affirms that restored channels intended as salmon rearing habitat must be designed to maintain sufficient depth during high tides for salmon access. Although higher elevation tidal channels may support and export salmon prey to other areas of the estuary, they likely will not be used by salmon directly.

Based on the timing of movement that we observed, tide gates also are likely to inhibit salmon movement if they remain closed or alter tidal flow at any time during the period beginning roughly 4 h before and ending roughly 6 h after high tide. Thus, although many recent restoration efforts have modified tide gates to improve passage of adult and juvenile fish (Giannico and Souder 2004), it is likely that any gate that functions to limit tidal flooding (the fundamental purpose of tide gates) will negatively affect access to habitat by juvenile salmon. The small proportion of tagged fish that entered our channel during the ebbing tide, however, suggests that salmon may opportunistically access habitats above tide gates if gates open early enough and remain open for a sufficient period during ebb tides.

Bottom et al. (2005*a*) provided evidence that restoration of marsh habitat in the Salmon River estuary has expanded expression of estuarine-rearing life histories within the contemporary Chinook salmon population. Juvenile Chinook salmon now enter the ocean at a broader range of sizes and times compared with a period prior to marsh restoration. Such behavioral diversity may spread the risks posed by variable oceanic or climatic conditions and increase resilience of the salmon population. The individual-based approach in this study reveals on a fine scale the ways in which juvenile Chinook interact with and exploit intertidal wetland habitat and affirms that a diversity of marsh rearing patterns exists in the present Chinook salmon population. The behavior of individuals on this finer scale is the mechanism that leads to the population-scale responses to restored habitat structure observed by Bottom et al. (2005*a*).

Preserving connectivity of intertidal marsh habitats within the estuary is critical to maintaining expression of behavioral diversity in estuarine rearing salmon. We suggest that future marsh rearing studies incorporate multiple PIT detection sites to examine the patterns of habitat use by individuals across the estuarine landscape and the connections among habitats that support diverse salmon rearing and migration behaviors.

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The stability and resilience of management agreements on climate-sensitive straddling fishery resources: the blue whiting (*Micromesistius poutassou*) coastal state agreement

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Abstract: How would the formation, stability, and success of an agreement on cooperative management between neighbouring coastal states for a climate-sensitive fishery resource be affected by changes in the distribution and accessibility of the resource within the exclusive economic zones (EEZs)? In scenario 1, the blue whiting (*Micromesistius poutassou*) is harvestable in the EEZs of Norway, Iceland, the Faroe Islands, and the European Union (EU), as well as in the international waters of the Northeast Atlantic and the Norwegian Sea. The Barents Sea is a fringe area for the species, and there are no fisheries for blue whiting there. Hence, Russia is not regarded as a coastal state with respect to the blue whiting fishery. This severely weakens the stability of the coastal state agreement. In scenario 2, the area of distribution of the harvestable stock expands into the Russian EEZ, giving it status as a coastal state with respect to the resource and, thus, a partner in the management agreement. This secures the coastal state coalition the maximum attainable cooperative value and increases the likelihood of a stable coastal state agreement.

Résumé : Comment l'établissement, la stabilité et le succès d'un accord sur la gestion en commun par des états côtiers limitrophes d'une ressource halieutique sensible au climat sont-ils affectés par les changements dans la répartition et l'accessibilité de la ressource dans les zones économiques exclusives (EZZ)? Dans un premier scénario, le poutassou (*Micromesistius poutassou*) est récoltable dans les EZZ de Norvège, d'Islande, des îles Féroé et de l'Union Européenne (EU), de même que dans les eaux internationales du nord-est de l'Atlantique et de la mer de Norvège. La mer de Barents représente une zone marginale pour l'espèce et il ne s'y fait pas de pêche de poutassous. La Russie n'est donc pas considérée comme état côtier en ce qui concerne la pêche aux poutassous. Ce scénario affaiblit considérablement la stabilité de l'accord entre les états côtiers. Dans le second scénario, l'aire de répartition du stock récoltable s'étend dans la EZZ russe, ce qui donne à la Russie le statut d'état côtier en ce qui a trait à cette ressource et en fait un des partenaires de l'accord de gestion. Ce scénario procure à la coalition d'états côtiers les conditions maximales possibles de coopération et augmente la probabilité d'un accord stable entre les états côtiers.

[Traduit par la Rédaction]

Introduction

How would the formation, stability, and success of an agreement on cooperative management between neighbouring coastal states for a climate-sensitive fishery resource be affected by changes in the distribution and accessibility of the resource within the exclusive economic zones (EEZs) of these states? A shift in the climate regime could have two types of effects on a climate-sensitive fishery resource: (*i*) changed biomass and (*ii*) changed area of distribution and (or) migration pattern. Of course, both are possible and closely linked. If the stock decreases in numbers, this will have a corresponding effect on distribution. Abundance changes may also have a density-dependent effect on catchability. For the purpose of this paper, it is the distribution and accessibility changes that are important, not necessarily what causes them.

Though it is a challenging task to achieve efficient management of a fishery that is confined to a single jurisdiction (Gordon 1954), further complications emerge when the targeted fish population migrates across international boundaries or straddles the boundary between a national jurisdiction and the international waters of the high seas (e.g., Munro 1979, 1987). In the case of a fish population that migrates across international boundaries, harvesting in each jurisdiction affects the availability of fish in the other

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jurisdiction (Criddle 1996; McKelvey 1997). If these nations harvest the shared stock competitively, they will tend to waste its potential value. Recognising that possibility, they may attempt to work out a cooperative division of the harvest, but maintaining cooperation is difficult, and many international agreements have degenerated into mutually destructive fish wars. This instability appears to result from changes over time in the parties' incentives to cooperate (Miller 1996; McKelvey 1997). Uncertainty regarding the magnitude and sources of variations in fish stocks is another stumbling block to cooperative harvest management. The parties may have different information or beliefs about how the stock is changing, and they may have a strategic interest in concealing that information from one another, or in promoting a particular interest-laden interpretation of the biological facts (Miller 2000).

The key questions in this literature have been the distribution of benefits arising from cooperative versus noncooperative management and stability of cooperative arrangements (Sandberg 2005). The latter typically demands analysis of incentives of individual players to defect from cooperation, as well as credible punishment of defectors by remaining players. When expected costs of a credible punishment are higher than expected benefits of defecting from a cooperative agreement, the agreement is self-enforcing, and vice versa. Lindroos and Kaitala (2001) provide an overview of this literature.

The likelihood of reaching a cooperative management of transboundary fishery resources thus depends on numerous factors. Munro (1979) was among the first to recognise that when fish stocks are transboundary, different regulatory bodies may have diverging social rates of discount, fishing effort costs, or consumer tastes. In the absence of side payments, such differences will imply that the various parties' views on optimal management will differ. Kaitala and Lindroos (2004) showed that timing may be important for whether or not to cooperate.

Hannesson (1997) analysed which factors tend to sustain cooperative management and found that a low (high) discount rate, a small (large) number of parties and a high (low) cost of fishing would make cooperative management more (less) likely. Several papers have dealt with how (limited) access may influence incentives to cooperate among the parties harvesting a fish stock. Assuming a uniformly distributed stock, Hannesson (1997) illustrates two different cases. In one of these, each identical player has sole access to a share of the area where the stock is distributed. This condition reflects a stock that is distributed across the EEZs of more than one nation, where each nation has exclusive access to harvest the resource in its own zone. Assuming limited migration of the stock between areas, he finds such designated access rights to increase the likelihood of cooperation relative to a stock where all players have access to the stock over its entire area of distribution. In the second case, a dominant agent has access to the largest area of stock distribution, whereas a competitive fringe controls the remaining area. In this case, he finds that the existence of a competitive fringe reduces the optimal stock level, as well as the corresponding net present value (NPV) of the fishery.

Arnason et al. (2000) discuss the benefit of cooperation through the use of a surplus production model in which prices are constant and harvest costs depend on the stock, harvest, and distance to the exploitable biomass. They investigate a number of different noncooperating coalitions that only have access to the stock on the high seas and in their own EEZs. In this way, limited access influences the incentives to cooperate via the cost function.

The inherent difficulty of managing such resources suggests that adaptation to the effects of climate variability and climate change is likely to be less complete and effective than might be the case for resources that are controlled by private property owners. Furthermore, climatic variations may destabilize efforts to cooperatively manage resources that are shared among multiple jurisdictions.

Work that has been done on the implications of climate regime shifts for fisheries management has largely been on fisheries confined within the EEZ of one, or at most two, coastal states (Hannesson et al. 2006). Miller and Munro (2004) (see also Miller (2000) and Miller et al. (2001)) presents evidence for the significance of climatic regime shifts and draws on the history of conflict between Canada and the United States over Pacific salmon (*Oncorhynchus* spp.) management to illustrate the dangers that unpredicted, unanticipated environmental regime shifts pose for the efforts to maintain international cooperation (Hannesson 2007). Another, well-documented example of this is the coastal state agreement on the management of the Norwegian spring-spawning herring (*Clupea harengus*) that was suspended for two years (2003 and 2004) (Hannesson 2006) when the stock failed to resume its expected migration pattern by spending the winter in Norwegian coastal waters rather than out in the open Norwegian Sea. The Norwegian fishermen, in particular, were not content with their share of the catches as the stock spent most of its time within the Norwegian EEZ (Sissener and Bjørndal 2005). Further, Miller (2007) examined the effects of climate variability on a small number of economically important shared and highly migratory marine fish resources and outlined the results of previous case studies that highlight the disruptive effects of climate variability on international harvest management efforts. In these cases, limited understanding and poor predictability of the biological impacts of climate variability contributed to the dysfunction or breakdown of existing cooperative management arrangements. Changing patterns of abundance and availability also led to shifts in power relationships among the competing harvesting entities. Furthermore, Miller (2007) then explored the potential management challenges posed by the effects of climatic variability on tropical tuna stocks in the western and central Pacific and western Indian oceans in light of the rapid development of commercial fisheries for tropical tuna in those regions over the past two decades. The fact that coastal states' EEZs encompass only part of the migratory range of the target fish, as well as the significant role of distant water fishing nations (DWFNs) in harvesting these stocks, creates an incentive structure that is quite different from the previously described cases.

In this paper, we examine, within the framework of noncooperative, endogenous formation of coalitions and coalition structures (Pintassilgo 2003; Lindroos et al. 2007), the effects of distribution scenarios on a management agreement between the coastal states with EZZs in which the blue whiting stock (*Micromesistius poutassou* Risso) in the Northeast Atlantic is harvestable. In addition, the blue whiting stock is harvestable on the high seas (international waters), where it is accessible not only to the coastal states, but also to vessels from DWFNs. The analysis is conducted, drawing on the games and model described in Ekerhovd (2008), by changing the shares (quarterly zonal attachment) of the blue whiting stock available in the different EEZs and the high seas.

In the past and at present, the blue whiting is harvestable in the EEZs of Norway, Iceland, the Faroe Islands, and the European Union (EU), as well as in the international waters of the Northeast Atlantic and the Norwegian Sea, beyond national jurisdiction. The Barents Sea is a fringe area for the species, and there are no fisheries for blue whiting there (Bailey 1982; Monstad 2004; Heino et al. 2008). Hence, Russia, although a major participant in the blue whiting fishery, is not regarded as a coastal state with respect to the blue whiting fishery.

This outlines the basis for the game analysed in Ekerhovd (2008) and one of the distribution scenarios analysed in this paper in which the coastal states formed a coalition maximizing their cooperative value given Russia's harvest in international waters. The main finding in Ekerhovd (2008) was that the benefits of the coastal state coalition are not sufficiently large to cover all member states' profits, simultaneously, to keep them from unilaterally leaving the coalition while the other coastal states remain in the coalition. However, if the option is either full costal state cooperation or no cooperation at all, the prospects for achieving and maintaining a coastal state coalition is brighter.

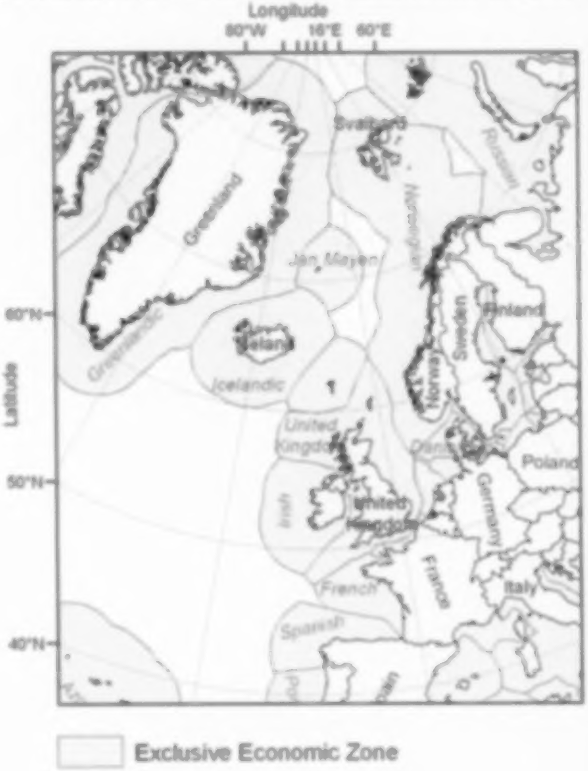
Another distribution scenario analysed here is a climatic regime in which the ocean temperature becomes warmer and the area of distribution of the harvestable blue whiting stock expands into the Russian EEZ, which would give it status as coastal state with respect to the resource and, thus, a partner in the management agreement. The inclusion of Russia as a coastal state will secure the maximum attainable cooperative value for the coastal state coalition. Whether or not the coalition becomes more stable depends on the extent to which the increase in coalition value is sufficient to compensate unilaterally the free-rider values of both Russia and the other coastal states. By free-riding, we mean someone who reaps (some of) the benefits of a cooperative arrangement without participating in it.

The blue whiting fishery

The blue whiting stock migrates through the EEZs of the EU, the Faroe Islands, Iceland, and Norway (Fig. 1), considered as the coastal states with respect to the stock, and into the international waters beyond the EEZs (Fig. 1), where it can be harvested by vessels from any country, not just the coastal states. Besides the coastal states, Russia is an important player in the blue whiting fishery.

After 1991, the landings of blue whiting steadily increased, until they suddenly increased from about 650 thousand tonnes in 1996 to 1.1 million tonnes in the next year and continued increasing from then on more or less steadily to about 2.4 million tonnes in 2004 (International Council for the Exploration of the Sea (ICES) 2007). To be able to fish blue whiting in the waters of other countries, the nations have negotiated bilateral quotas within the various zones. Because of the lack of agreed sharing of the quota, the negotiations did not consider the recommended total allowable catch (TAC). In addition, each country allowed for unlimited landings from its own waters, as well as from international waters. As a result, the actual harvest in 2001 was in fact almost three times as large as recommended by ICES (2003).

Fig. 1. The exclusive economic zones (EEZs) and the high seas (North East Atlantic Fisheries Commission (NEAFC) regulatory area) in the Northeast Atlantic (NEAFC convention area). Source: Institute of Marine Research, P.O. Box 1870 Nordnes N-5817 Bergen, Norway, with EEZs from Flanders Marine Institute (Belgium).



As the landings of blue whiting grew to significant quantities, it became clear that an international agreement was needed on how to share this resource among the nations involved. The North East Atlantic Fisheries Commission (NEAFC) organized a series of meetings to this end, including workshops, discussions, and negotiations. However, despite two years of such meetings, in the early 1990s, when the matter was thoroughly dealt with, no agreement was reached on how to share the TAC, i.e., the quota recommended by NEAFC on the basis of advice from ICES (Monstad 2004; Bjørndal 2009).

NEAFC is a regional fisheries management organization with membership open to all parties with real interests in the fish stocks within the areas covered by the convention. NEAFC is intended to serve as a forum for consultation, the exchange of information on fish stocks, and the management of these and to advise on the fisheries in the high sea areas mentioned in the convention on which the commission is based. Because most of the fisheries take place within the jurisdiction of the coastal states, NEAFC has no real management responsibilities beyond the fraction of the fish stocks located within the high seas areas covered by the convention (Bjørndal 2009).

However, in December 2005, the coastal states consisting of the EU, the Faroe Islands, Iceland, and Norway signed an agreement (Appendix A). The agreement, starting in 2006, includes a long-term management strategy that implies annual reductions in the landings until the management goals are reached (Anonymous 2006). This arrangement provided for catches in 2006 of 2 million tonnes, allocated as follows: the EU, 30.5%; the Faroe Islands, 26.125%; Norway, 25.745%; and Iceland, 17.63%. Russia will be accommodated by quota transfers from some of the coastal states and additional catches in the NEAFC area (Appendix B).

Although it would be interesting to explicitly examine the possible role of the practice of bilateral quota swapping in maintaining cooperation when it would otherwise be difficult to sustain, we do not consider arrangements that allow foreign vessels to fish blue whiting inside other nations' EEZs. The quota-swapping bargaining involves exchanging quotas on several different fish species, and in a proper analysis of these practices, several fisheries should be considered simultaneously. While acknowledging the importance of bilateral arrangements, we stylize the blue whiting management and assume that the fleets fish in their respective EEZs and in the high seas areas, the NEAFC regulatory area (NEAFC RA).

Material and methods

Distribution of the blue whiting stock

The year (y) is divided into quarters, j, and i denotes the respective fleets (for a list of symbols, see Table 1). In the noncooperative case, the is are identical to the EEZs of the coastal states, and NEAFC RA (Bjørndal 2009) are the international waters (Fig. 1). Thus, $\theta_{i,j}$ denotes the shares of the blue whiting stock available for harvest in the different waters throughout the year. Harvest within a certain year is modelled sequentially. That is, the blue whiting stock migrates through different waters during a year (Fig. 2) and is available for harvest in different proportions in the EEZs and the high seas areas in the Northeast Atlantic, depending on the season. When the blue whiting is not present in a coastal state's EEZ, the fleet from that country can only fish blue whiting in international waters, if possible. This is a simplification that we make. In reality, bilateral agreements exist allowing foreign vessels access to the stock in national waters. Otherwise, they can harvest in their home waters, as well as on the high seas.

The Norwegian Sea and the Barents Sea area is one of the world's richest, purest, and most productive marine areas and is one where the climate, in both the sea and the atmosphere, is expected to change in response to global warming (Arctic Climate Impact Assessment (ACIA) 2004; Stenevik and Sundby 2007; Ellingsen et al. 2008).

As the water temperature rises, the blue whiting's distribution boundaries will probably be extended to the north and east, and it is likely to occur in the area in higher concentrations and more regularly than in the past. Eventually, the blue whiting will possibly inhabit the southwestern parts of the present Arctic area on a permanent basis (ACIA 2004).

However, there is considerable uncertainty about the realisation of the above scenario, and the effects of climate change on the blue whiting in the Barents Sea might be small or insignificant. Simulations show that there will be no change in the decadal mean flow of water into the Barents Sea during the next 50 years, but the temperature of the water transported into the Barents Sea will become significantly higher (increase of about 1 °C during the period) (Ellingsen et al. 2008). Even though the simulation results show that the future climate will be warmer, there are also years when the climate is comparable to periods of the present climate. The position of the Polar Front in the Barents Sea, separating the warm Atlantic water from the cold Arctic water, is strongly governed by topography in the western part of the Barents Sea. In the east, where the topographic control is weak, changes of the position of the Polar Front towards the north and east are more likely (ACIA 2004).

The blue whiting stock increased from 1995 to 2004, when it started to decline. Simultaneously to the increased stock size, the stock expanded its horizontal distribution area towards north and west. When the stock started to decline again, it remained in the northern and western areas (K.R. Utne (Institute of Marine Research, Nordnesgt. 33, 5085 Bergen, Norway, kjell.rong.utne@imr.no), G. Huse, G. Ottersen, V. Zabavnikov, J.A. Jacobsen, G.J. Óskarsson, and L. Nøttestad, unpublished data).

Hátún et al. (2009*b*) documented a link between a weak subpolar gyre (Hátún et al. 2005) and strong blue whiting recruitment, along with a shift in spawning area, from south to north, i.e., from the Porcupine Bank southwest of Ireland to the Rockall Plateau west of the Hebrides (Salthaug 2009). Furthermore, Hátún et al. (2009*a*) showed that during the warm and saline condition, associated with a weak subpolar gyre index, the blue whiting fishery was more spatially distributed towards the western areas in Icelandic and Faroese waters than in colder and less saline periods. This indicates that under warmer climatic conditions, blue whiting distribution does not necessarily, or at least not only, expand its boundaries to the north and east in the Barents Sea, but rather to the north and west in the Norwegian Sea.

Here we present some potentially plausible relative shares (Tables 2 and 3) that we have postulated. It is difficult to determine the relative shares empirically, especially scenario 2, as a situation in which Russia is able to harvest blue whiting in its EEZ and is regarded as coastal state by the others has not been observed. Therefore, the shares are used illustratively and intended only to represent possible scenarios.

Scenario 1

Scenario 1 (Table 2) denotes the case of a more westerly distribution of the blue whiting stock in the Norwegian Sea. Spawning takes place in the waters between Iceland and the Faroe Islands, as well as in EU waters. The westerly distribution reduces the availability of the blue whiting in interna-

Table 1. List of symbols and abbreviations.

	Definition	Range	Unit	Subscript
Subscripts				
a	Age	[1, 2, …, 10+] years		
y	Time	Current year		
j	Quarter	[1, 2, 3, 4]		
i	Fleet index	n		
n	Number of fleets	Fleets		
Variables				
N	Abundance		Numbers	a, j, y
SSB	Spawning stock biomass		kg	y
R	Recruitment		Numbers	y
X	Fishing effort			i
Π	Profit		NOK	i,j,y
V	Value		NOK	i
P	Coalition value		NOK	i
F	Fishing mortality			a,j,i
Parameters				
m	Natural mortality		0.2	a
p	Price		0.8 NOK	
r	Discount rate		5%	
θ	Zonal attachment		cf. Tables 2 and 3	i,j
mo	Maturity ogive		cf. Table 4	a
q	Accessability		cf. Table 4	a,j
w	Weight		cf. Table 4	a
α	Recruitment parameter		cf. Table 5	
β	Recruitment parameter		cf. Table 5	
c	Cost parameters		cf. Table 6	i
Abbreviations				
ACIA	Arctic Climate Impact Assessment			
NEAFC	North East Atlantic Fisheries Commission			
RA	Regulatory area			
ICES	International Council for the Exploration of the Sea			
ACFM	Advisory Committee on Fisheries Management			
NOK	Norwegian kroner			
CS	Coastal states			
C	Coalition structure			
S	Coalition			
DWFN	Distant water fishing nation			
TAC	Total allowable catch			
EU	European Union			
FO	Faroe Islands			
IS	Iceland			
NO	Norway			
RU	Russia			
NPV	Net present value			

tional and Norwegian waters, and Russia is not regarded as a coastal state.

The blue whiting stock becomes more evenly distributed within the initial coastal states' EEZs, and between the EEZs and the high seas. Furthermore, Russia is excluded from participating in the coastal state coalition, as in Ekerhovd (2008).

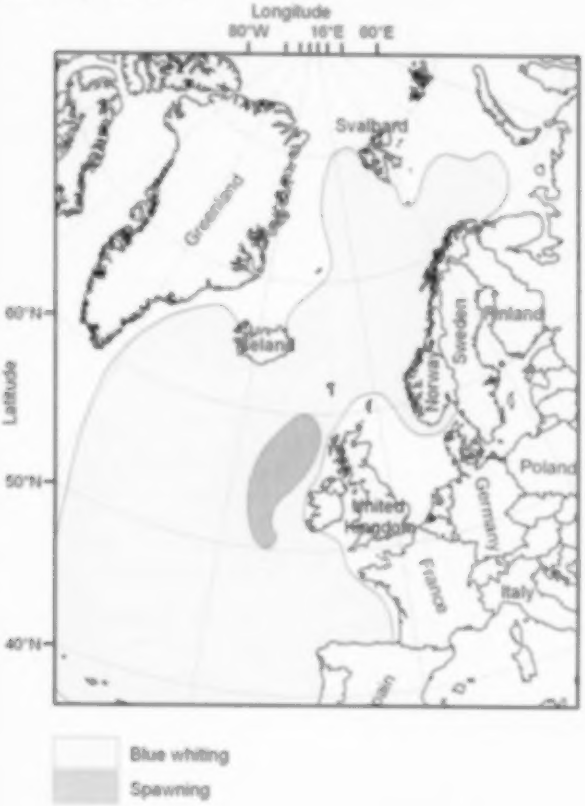
Scenario 2

Scenario 2 (Table 3) denotes the case in which the habitat of the blue whiting expands northeastward into the Barents Sea such that Russia becomes a coastal state and the blue whiting appears in Norwegian waters during the second quarter, in addition to EU and Faroese waters.

Biological submodel

Our model encompasses age groups 1 to 10+. The age groups are harvested simultaneously by applying a fleet-specific fishing mortality $F_{a,j,i}$ to all age groups. The catch rate for each fleet i is governed by two parameters, the effort, X_i, and the accessability coefficient, $q_{a,j}$, where a denotes the age group and j denotes the season. This is a

Fig. 2. Map summarizing the migration pattern and areas of concentration of adult blue whiting (*Micromesistius poutassou*). Source: Institute of Marine Research, P.O. Box 1870 Nordnes N-5817 Bergen, Norway.



version of the classical Schaefer (1957) production function, which assumes proportionality between effort and fishing mortality.

The selectivity of the pelagic trawls used in the blue whiting fishery is, at least in principle, uniform for all age groups, meaning that the gear catches fish indiscriminately of size or age. The reason for this lack of age-specific escapement from the gear is that in the opening of the trawl, which covers a huge area of water (Standal 2006), the mesh size is quite large, several metres in fact (Bailey 1982), and at the other end, where the fish finally end up, the mesh size is much smaller, about 50 mm. Furthermore, there are one or two extra nets outside the fish end to prevent it from breaking as a result of the increased pressure generated when the swim bladder expands as the fish is forced to the surface (Monstad 2004).

Table 2. Scenario 1 — quarterly zonal attachment, $\theta_{i,j}$, of the blue whiting (*Micromesistius poutassou*) stock.

	Quarter			
	1	2	3	4
Scenario 1*a*				
NEAFC RA	1/2	0	1/6	1/6
EU	1/2	1/2	0	0
FO	0	1/4	1/3	1/3
IS	0	1/4	1/3	1/3
NO	0	0	1/6	1/6
RU	0	0	0	0
Scenario 1*b*				
NEAFC RA	1/2	0	1/4	1/4
EU	1/2	1/2	0	0
FO	0	1/4	9/32	9/32
IS	0	1/4	9/32	9/32
NO	0	0	3/16	3/16
RU	0	0	0	0
Scenario 1*c*				
NEAFC RA	1/2	0	1/4	1/4
EU	1/2	1/4	0	0
FO	0	1/4	1/4	1/4
IS	0	1/4	1/4	1/4
NO	0	1/4	1/4	1/4
RU	0	0	0	0

Note: See Table 1 for abbreviations.

Table 3. Scenario 2 — quarterly zonal attachment, $\theta_{i,j}$, of the blue whiting (*Micromesistius poutassou*) stock.

	Quarter			
	1	2	3	4
Scenario 2*a*				
NEAFC RA	1/3	0	1/3	1/3
EU	1/3	1/3	0	0
FO	1/9	1/3	1/9	1/9
IS	1/9	0	1/9	1/9
NO	1/9	1/3	1/3	1/3
RU	0	0	1/9	1/9
Scenario 2*b*				
NEAFC RA	1/2	0	1/4	1/4
EU	1/8	1/3	0	0
FO	1/8	1/3	1/6	1/6
IS	1/8	0	1/6	1/6
NO	1/8	1/3	1/4	1/4
RU	0	0	1/6	1/6
Scenario 2*c*				
NEAFC RA	1/4	0	1/5	1/5
EU	1/4	1/4	0	0
FO	1/6	1/4	1/5	1/5
IS	1/6	1/4	1/5	1/5
NO	1/6	1/4	1/5	1/5
RU	0	0	1/5	1/5

Note: See Table 1 for abbreviations.

The abundance of each age group in landings from specific areas varies over time and is governed by many factors. The age distribution of the landings is not uniform across the age groups. Instead, we stylize the accessibility coefficients based on assumptions about the age distribution for each area that seems reasonable (Table 4). In the first two quarters of the year, the stock is either migrating towards or already in the spawning areas (Fig. 2). Therefore, the accessibility coefficients for quarters 1 and 2 are set equal to the age-specific proportion of the maturity ogive, that is, the age distribution of the harvest is equal to the age distribution in the spawning stock biomass (SSB). In quarter 3, the stock

Table 4. Blue whiting (*Micromesistius poutassou*): proportion of maturation, accessibility, weight at age ($kg \cdot individual^{-1}$), and numbers at age (in millions) in 2000 and 2006.

	Accessibility coefficients				Numbers at age	
Age group	mo_a*	$q_{a,3}$	$q_{a,4}$	Weight at age	2000	2006
1	0.11	1.00	1.00	0.049	39 743.1	1 1141.0
2	0.40	1.00	1.00	0.075	16 963.6	18 435.0
3	0.82	1.00	1.00	0.102	16 123.1	18 369.9
4	0.86	1.00	1.00	0.125	12 150.7	15 955.9
5	0.91	0.50	1.00	0.147	3 813.6	7 862.8
6	0.94	0.25	1.00	0.168	909.8	5 220.1
7	1.00	0.10	1.00	0.185	435.0	1 440.2
8	1.00	0.10	1.00	0.200	207.4	337.0
9	1.00	0.10	1.00	0.222	138.7	209.3
10+	1.00	0.10	1.00	0.254	384.3	171.1

*Maturity ogive, used as an age-specific accessability coefficient in catches of the first and second quarters, $q_{a,1}$ and $q_{a,2}$, respectively.

has finished spawning and has migrated to the feeding areas in the Norwegian Sea. As the older individuals start the migration earlier and travel farther than the younger ones (Bailey 1982), they are too widely dispersed on their migration to be caught. Moreover, younger individuals are reported as being over-represented in the landings from the Norwegian Sea during summer (Heino 2006). Furthermore, the accessibility coefficients of quarter 3 are set to unity for the younger age groups, while being held at a lower level for the older ones. In quarter 4, we assume that the entire stock congregates before starting the migration back to the spawning grounds. This results in a uniform age distribution. Note that the $q_{a,j}$s distribute the overall fishing effort across the different age groups (Table 4).

All age classes are subject to natural mortality, *m*, set to 0.2 for all age groups, which appears to be a common assumption and used for stock assessment (ICES 2007). It is assumed that only the older component of the population (from age class 7 on) is fully mature, whereas the younger age classes are only partially mature. The values for the maturity ogive (Table 3) were estimated by the 1994 Blue Whiting Working Group (ICES 1995). The estimate of the maturity ogive defines the proportion of the mature individuals in the age class as constant average, mo_a, for each age class. The annual SSB is then given by

$$(1) \qquad SSB_y = \sum_{a=1}^{10+} mo_a w_a N_{a,y}$$

where *w* is the individual weight (in kilograms; Table 4; ICES 2007), and *N* is the number of fish.

The stock in the beginning of the first quarter of each year is equal to the recruitment to the youngest cohort plus the fish that survived the last quarter of the previous year.

To determine a possible stock–recruitment relationship, we try to fit the well-known stock–recruitment models of Beverton and Holt (1957) and Ricker (1954) to data on the SSB and recruitment. However, we failed to obtain any satisfactory fit between the data and the models (Ekerhovd 2008). Instead, a serially correlated recruitment relationship, estimated on the recruitment from 1981 to 2006, reported in ICES (2007), was used in linking the number of recruits, R_y, to the previous year's recruitment, R_{y-1}. An explanation for this relationship is that the recruitment is mainly dependent on various environmental factors such that a possible stock–recruitment relationship drowns in the noise (Ekerhovd 2008). In addition, the serial correlation that we found indicates that good or bad environmental conditions occur at least two years in a row (Fig. 3).

When running this serially correlated recruitment process, starting from any initial recruitment level, the recruitment will converge to a certain recruitment level given the parameter values, and this level is independent of the fishing effort applied. This means that the steady-state recruitment of the serially correlated recruitment process (Table 5) will be about 21.5 billion individuals entering the fishable stock in steady state (Fig. 4). This recruitment level is relatively strong if we compare it with the average recruitment of the period 1981–1995, which was less than 10 billion recruits, but moderate if we compare it with the average recruitment of about 36 billion for the years 1996–2005. Such a strong and reliable recruitment would lead to an unrealistic and overoptimistic valuation of the stock and leave us with the impression that the stock can sustain a very high fishing effort indefinitely. To compensate for this and in spite of the fact that we were unable to establish any stock–recruitment relationship, we let the recruitment process be dependent on the SSB, as follows.

In 1998, ICES's Advisory Committee on Fisheries Management (ACFM) defined limit (lim) and precautionary (pa) reference points for this stock as follows: B_{lim} (1.5 million tonnes), B_{pa} (2.25 million tonnes), F_{lim} (0.51), and F_{pa} (0.32) (ICES 1998). The advice of ACFM in the following years has been given within a framework defined by these reference points (ICES 2003).

Note that we do not treat the reference points as something that the countries have agreed upon (Lindroos 2004), but rather as a biological feature of the stock, and that fishing could continue even when the spawning stock is below B_{lim}.

As long as SSB is greater than or equal to B_{pa}, we let the recruitment follow the serially correlated process $R_y = \alpha +$

Fig. 3. Blue whiting (*Micromesistius poutassou*) recruitment (in millions) observed (◆) and estimated (☐) using the serial recruitment relationship $R_y = \alpha + \beta \times R_{y-1}$, plotted over time, 1981–2006 (ICES 2007).

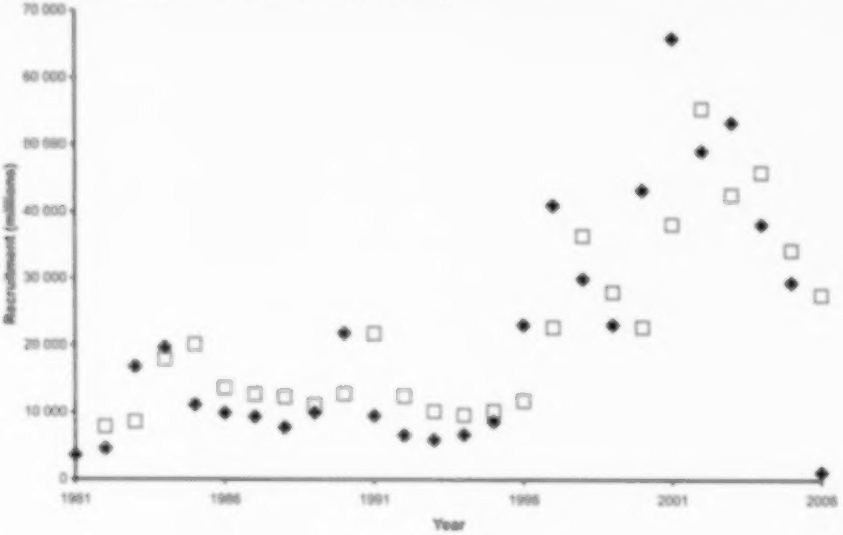


Table 5. Estimated serially recruitment relationship fitted to data from 1981–2006 (ICES 2007).

Parameters			
α	β	R^2_{adjusted}	Durbin–Watson test statistic
5113.6 (3790.4)	0.76 (0.14)	0.56	1.51

Note: Standard errors in parentheses.

$\beta \times R_{y-1}$. If SSB falls below B_{pa} but stays above B_{lim}, the recruitment is fixed at α and 5113.6 million individuals are recruited annually. Further reduction of SSB below B_{lim} leads to partial recruitment failure, with recruitment dropping to only 500 million recruits annually. Hence,

$$(2) \qquad R_y = \begin{cases} 500 & \text{if } \mathrm{SSB}_{y-1} < B_{\text{lim}} \\ \alpha & \text{if } B_{\text{lim}} \leq \mathrm{SSB}_{y-1} < B_{\text{pa}} \\ \alpha + \beta \times R_{y-1} & \text{otherwise} \end{cases}$$

The empirical foundation for what will happen to the recruitment if the SSB is severely reduced is weak. Over the period from 1981 to 2006 (Fig. 5), a SSB below B_{lim} has hardly been observed, was reported to be less than B_{pa} only a few times, and certainly did not collapse. Moreover, the period from 1996–2005 can be regarded as extraordinary. In hindsight, and in spite of the high and increasing fishing mortality of this period, the SSB is estimated to have been about 4.3 million tonnes in 2000, about 4.6 million tonnes in 2001, and increasing until at least 2003 (Fig. 5). However, evidence from other heavily exploited fish stocks suggests that sustained harvesting outside what is considered safe biological limits will eventually lead to recruitment failure and stock collapse, although under favourable environmental conditions, it may take some time for this to become evident. Hence, we have decided to follow the biologists in assuming that low SSB and high fishing mortality indicate that the stock is harvested outside safe biological limits that will eventually end in a recruitment failure.

The numbers of fish at the beginning of a season that have survived previous quarter's harvest and avoided death by natural causes are given as follows (dropping the year subscript y):

$$(3) \qquad N_{a,j} = N_{a,j-1}\left\{\sum_i \theta_{i,j-1}\,\mathrm{e}^{-[m/4+q_{a,i,j-1}X_i]} + \theta_{\text{NEAFC},j-1}\,\mathrm{e}^{-[m/4+q_{a,i,j-1}\sum_i X_i]}\right\}$$

Game theory

Before we continue with the economic model, we discuss equilibrium concepts and solution methods.

To predict the outcome of the game, we focus on the possible strategy profiles, as it is the interaction of the different players' strategies that determines what happens. The distinction between strategy profiles, which are sets of strategies, and outcomes, which are sets of values of whichever variables are considered interesting, is a common source of confusion (Rasmusen 2007). Often different strategy profiles lead to the same outcome.

Predicting what happens consists of selecting one or more strategy profiles as being the most rational behaviour by the players acting to maximize their payoffs.

Fig. 4. Development of the serially correlated recruitment relationship $R_t = \alpha + \beta \times R_{t-1}$ over time from a high 2001 (×) and a low 2006 (○) initial recruitment level, respectively.

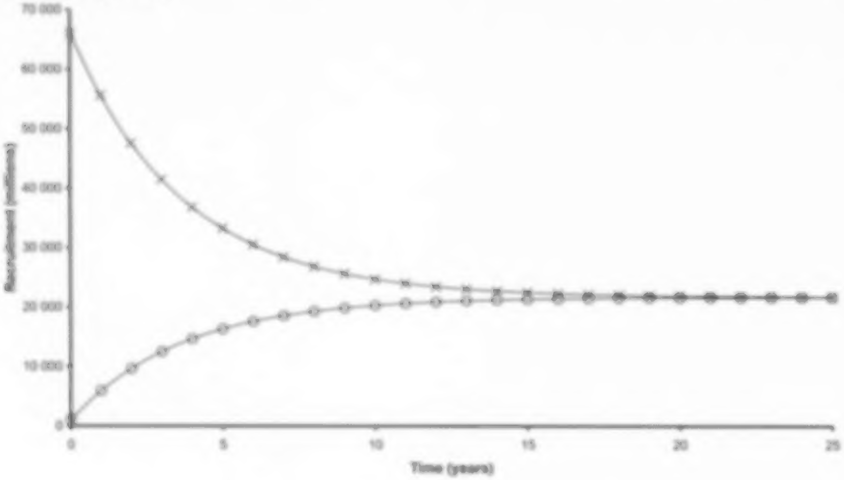
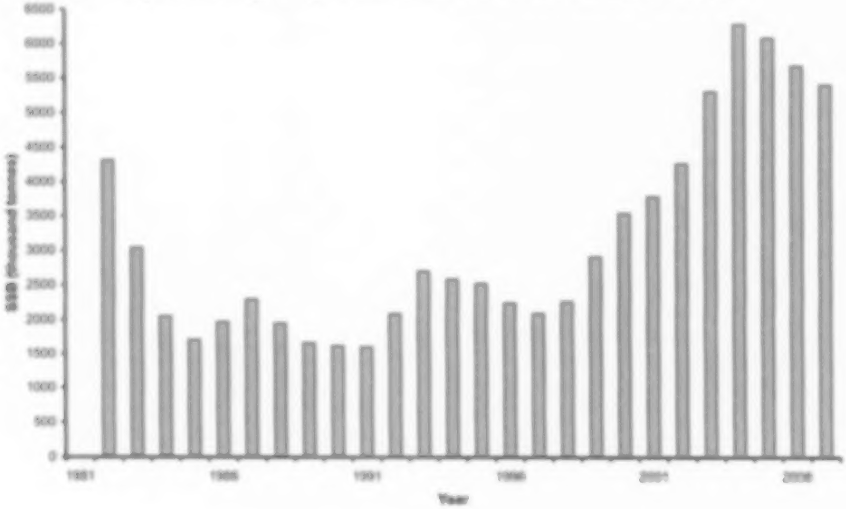


Fig. 5. Blue whiting (*Micromesistius poutassou*) spawning stock biomass (SSB), 1981–2006 (ICES 2007).



> An equilibrium $X^* = (X_1^*, \ldots, X_n^*)$ is a strategy profile consisting of a best strategy for each of the *n* players in the game. (Rasmusen 2007, p. 18)

The equilibrium strategies are the strategies that players pick in trying to maximize their individual payoffs, as distinct from the many possible strategy profiles obtainable by arbitrarily choosing one strategy per player. In game theory, the set of payoffs would be an equilibrium outcome, but the equilibrium itself would be the strategy profile that generated the outcome.

To find the equilibrium, it is not enough to specify the players, strategies, and payoffs, because we must also decide what "best strategy" means. We do this by defining an equilibrium concept.

> An equilibrium concept or solution concept $H(X_1, \ldots, X_n, V_1, \ldots, V_n) \rightarrow X^*$ is a rule that defines an equilibrium based on the possible strategy profiles and the payoff functions. (Rasmusen 2007, p. 18)

Accepted solution concepts do not guarantee uniqueness, and lack of a unique equilibrium is a major problem in game theory. Often the solution concept employed leads us to believe that the players will pick one of the two strategies A or B, but we cannot say whether A or B is more likely.

A model with no equilibrium or multiple equilibria is underspecified. The modeller has failed to provide a full and precise prediction for what will happen. One option is to admit that the theory is incomplete. Or perhaps the situation being modelled really is unpredictable, in which case to make a prediction would be wrong. Another option is to renew the attack by changing the game's description or the solution concept (Rasmusen 2007).

In discussing equilibrium concepts, it is useful to have a shorthand for "all other fleets' fishing efforts." For any vector $I = (I_1, \ldots, I_n)$, denote by I_{-i} the vector $(I_1, I_{i-1}, I_{i+1}, \ldots, I_n)$, which is the portion of I not associated with fleet i.

Using this notation, X_{-i} is the profile of fishing effort strategies for every fleet except for fleet i. That profile is of great interest to i, because it uses it to help choose its own fishing effort, and the new notation helps define its best response. Fleet i's best response to the fishing efforts X_{-i} chosen by the other fleets is the fishing effort X_i^* that yields it the greatest NPV, that is,

$$(4)\qquad V_i(X_i^*, X_{-i}) \geq V_i(X'_i, X_{-i}) \quad \forall \quad X_i' \neq X_i^*$$

The best response is strongly best if no other fishing efforts are equally good, and weakly best otherwise.

Nash (1951) equilibrium is the standard equilibrium concept in economics. Nash equilibrium is so widely accepted that the reader can assume that if a model does not specify which equilibrium concept is being used, it is Nash or some refinement of Nash.

> The fishing effort (strategy) profile is a Nash equilibrium if no fleet (player) has incentives to deviate from its fishing effort given that the other fleets do not deviate. (Rasmusen 2007, p. 26)

Formally,

$$(5)\qquad \forall i, \quad V_i(X_i^*, X_{-i}^*) \geq V_i(X_i', X_{-i}^*), \quad \forall X_i'$$

A straddling stock fishery usually involves many countries and fleets. The analysis of games in which the number of players exceeds two requires analysis of coalitions (Sumaila 1999). A coalition means a subset of the set of players. Two or more countries are considered to form a coalition if they ratify (or sign) a mutual agreement on the particular fishery. Three types of coalition scenarios may result. If all parties concerned sign the agreement, the situation is denoted full cooperation, and a grand coalition is said to be formed. If some countries are left outside the agreement, the situation is denoted partial cooperation, and the outsiders may act as free riders (Lindroos et al. 2007). Finally, in the case of noncooperation, there are no agreements between the countries, and each is only interested in maximizing individual net present value from the fishery. The value for coalition members depends on the particular behaviour of nonmembers. The assumption made in this paper is that nonmembers of the coastal state coalition act as single fleets (singletons) and adopt individually best-response fish efforts (strategies) against other fleets. This results in a Nash equilibrium between the fleets.

In a second stage, it is assumed that the members of the coalition act cooperatively, forming a single common fleet, by choosing a fishing strategy that maximizes the net present value for the coalition, given the strategies of the outsiders. The outsiders, or all players in the case of noncooperation, choose the strategy that maximizes their own individual net present values given the behaviour of the other players. This noncooperative behaviour leads to a noncooperative solution for each coalition structure (Lindroos et al. 2007).

The game is solved backwards. The result will be a Nash equilibrium of the game where it is not profitable for any of the coalitions or players to unilaterally deviate from the equilibrium strategies. The procedure for solving the game has to be repeated for each possible coalition structure. Having solved all these games allows us to proceed to stage 1 to find the equilibrium coalition structure. Of course, there may exist several equilibrium coalition structures, one equilibrium, or none at all.

In the blue whiting fishery game, each player simultaneously announces a list of players (including itself) with whom it is willing to form a coalition. The players that announce exactly the same list of nations belong to the same coalition. Given the players' announcements, the resulting coalition structure is one in which the players belong to the same coalition if and only if they choose exactly the same list of players (Yi 2003). Coalition structures C can be characterized as follows.

Let us assume the coastal states in scenario 1, consisting of the European Union (EU), the Faroe Islands (FO), Iceland (IS), and Norway (NO), with Russia (RU) as a DWFN. If the coastal states find it beneficial to form a coalition (EU,FO,IS,NO), while (RU) formulates its own unilateral fishing effort strategy, the coalition structure is (EU,FO,IS,NO),(RU). Clearly, the benefits of the coastal state coalition must depend on the fishing effort decision of Russia because externalities are present in fisheries (Pintassilgo 2003).

In scenario 2, on the other hand, the coastal states, now consisting of the European Union (EU), the Faroe Islands (FO), Iceland (IS), Norway (NO), and Russia (RU), find it beneficial to form a coalition (EU,FO,IS,NO,RU). Thus, the coastal states act as a cooperative unit, full cooperation is achieved, and (EU,FO,IS,NO,RU) is the resulting coalition structure.

If we compare the NPVs of the coastal state coalitions, we will typically find that it is larger in scenario 2 when all countries cooperate than in scenario 1, when Russia is outside the coalition, unilaterally maximizing its own NPV.

We can calculate the net present values for the coalition game setting. We do not, however, calculate the NPVs for every possible coalition structure of the game but restrict our analysis to calculate the NPVs of the coastal state coalition and the NPVs accruing to its members from unilateral free-rider behaviour. In addition, we calculate the individual NPV to players when all act noncooperatively.

For the singletons, we assume that the countries play a noncooperative game. This means that when a country does not belong to any coalition, it does not cooperate, and all that it can do is maximize its own profit, taking into account the strategies of the other players.

The countries outside the coalition will play noncooperatively against the coalition members. Thus, the members of the coalition will try to do their best, taking into account the actions of the outside countries and vice versa.

Under full cooperation, the value of the grand coalition, where all players are cooperating, is given by maximizing the sum of net revenues of the countries.

A coalition is said to be stable if there is no player that finds it optimal to join the coalition (external stability) and if no player within the coalition finds it optimal to leave the coalition (internal stability) (Lindroos et al. 2007).

When determining the stability properties of the coastal state coalition, it is sufficient to check for internal stability. To verify this, we can apply the criterion of stand-alone stability (Yi 1997), which states that a coalition is stand-alone stable if and only if no player finds it profitable to leave its coalition to form a singleton coalition, holding the rest of the coalition structure constant (including its former coalition). In the case of the coastal state coalition, this occurs when no coastal state is interested in leaving the coastal state coalition to adopt a free-rider behaviour.

In the management of shared and straddling fish stocks, positive externalities are expected to be present. In fact, as the members of a coastal state agreement tend to adopt strategies conserving the stock, a nonmember state is typically better off the greater the number of states that join the agreement. Pintassilgo (2003) established that the existence of externalities is a necessary, but not sufficient, condition for a coalition structure not to be stand-alone stable. To do so we define the coalition value.

> A game in partition function form specifies a coalition value $P(S,C)$ for every coalition structure and every coalition S that is an element of C. (Pintassilgo 2003, p. 179)

Formally, this can be written as:

$$(6)\qquad P(S,C) = V(S,C) - \sum_{i \in S} V(S^i, C_T)$$

where $P(S,C)$ denotes the net value of coalition S under the coalition structure C; $V(S,C)$, the NPV of coalition S under coalition structure C; S^i, a singleton coalition formed only by player i; and C_T, the coalition structure in which all players act as singletons. Therefore, $V(S^i, C_T)$ is the threat point of player i.

A sufficient condition for a coalition structure not to be stand-alone stable is that the sum of the coalition value of the singleton coalitions, resulting from unilateral deviations from any of its coalitions, exceeds the value of that coalition (Pintassilgo 2003).

Economics

ICES's ACFM Northern Pelagic and Blue Whiting Working Group has conducted surveys and published reports on the development of the blue whiting stock. Data available on the economics of the blue whiting fishery, on the other hand, are scarce, not at all structured, dispersed, and not consistent. The exception is the Norwegian revenue surveys, collected by the Directorate of Fisheries 1991–2004, in which data from vessels targeting blue whiting along with several other important species are published (Ekerhovd 2007). Because of the severe data constraints, we build the model and determine intuitively those parameters that cannot be estimated for lack of data.

The profit earned by fleet i during a quarter of the year is as follows (dropping the year subscript y):

$$(7)\qquad \Pi_{i,j} = pX_i \sum_{a=1}^{10+} q_{a,j} N_{a,j} w_a \left[\frac{\theta_{i,j}\left(1 - e^{-(m/4 + q_{a,j}X_i)}\right)}{m/4 + q_{a,j}X_i} + \frac{\theta_{\mathrm{NEAFC},j}\left(1 - e^{-(m/4 + q_{a,j}(X_i + X_{-i}))}\right)}{m/4 + q_{a,j}(X_i + X_{-i})} \right] - c_i X_i/4$$

The first line of the right-hand side of eq. 7 is simply the price per unit, p (because most of the blue whiting landed is used for low-priced products such as fish meal and fish oil, the price is set equal to 0.8 NOK·kg^{-1}), multiplied by the weight of fleet i's quarterly harvest of all age groups, where w_a denotes the individual fish weight at age (Table 4). Again, note that X_i denotes fleet i's fishing effort, $q_{a,j}$, the quarterly age-specific accessibility coefficients, and $N_{a,j}$, the number of fish in age group a at the beginning of quarter j that have survived the previous quarter's harvest and avoided death by natural causes (cf. eq. 3). The first expression inside the brackets is the share of the total quarterly mortality from fishing in fleet i's EEZs, where $\theta_{i,j}$ denotes the shares of the total stock occupying the respective fleet's (coastal states) EEZs in quarter j, $m/4$ is the quarterly natural mortality, and $q_{a,j}X_i$ is the age-specific quarterly fishing mortality produced by fleet i's fishing effort only. Likewise for the second expression inside the brackets, but here $\theta_{\mathrm{NEAFC},j}$ denotes the share of the total stock occupying the high seas inside NEAFC convention area (Tables 2 and 3). In these waters (the NEAFC RA), fishing is open to all fleets. Therefore, the total age-specific quarterly fishing mortality here, $q_{a,j}(X_i + X_{-i})$, is a function of the sum of all fleets fishing effort (X_{-i} is the other fleet's fishing efforts). The expression on the second line is fleet i's total quarterly costs, where c_i is fleet i's cost parameter (Table 6), for every coalition structure and zonal attachment distribution in the game.

Here X is purely notational, and the only modes of cooperation observed are where the countries compete against each other, i.e., no cooperation at all, or full cooperation among the coastal states, with Russia as a nonmember. However, there are several possible ways in which the countries can engage in partial cooperation that are not observed in real life. Nevertheless, these intermediate and hypothetical levels of cooperation are important in finding the Nash equilibrium in a coalition game. Hence, to be able to proceed with this analysis, we need a consistent method of finding cost parameters for every coalition under every imaginable coalition structure. This method is as follows. Assume that all fleets apply an effort, X^{∞}, that results in a minimum recruitment such that the minimum stock level is reached after 35 years. The resulting cost parameters (Table 6) were set such that the present value of the revenues was absorbed by the costs. Our goal here is not to find the intramarginal profit of open access based on rational behaviour but intuitively determine those coefficients that cannot be estimated for lack of data.

The NPV of fleet i as a function of the control variable X_i is given by

Table 6. Coalition cost parameter (in million NOK).

	X^*	CS	EU	FO	IS	NO	RU
Scenario 1*a*							
(EU,FO,IS,NO,RU)	0.1301	6735					
(EU,FO,IS,NO),(RU)	0.104	5776					1953
(EU,FO,IS,RU),(NO)	0.104	4416				3314	
(EU,FO,NO,RU),(IS)	0.104	5609			2121		
(EU,IS,NO,RU),(FO)	0.104	5178		2552			
(FO,IS,NO,RU),(EU)	0.104	5223	2507				
(EU),(FO),(IS),(NO),(RU)	0.0655		2387	2460	2054	3243	1894
Scenario 1*b*							
(EU,FO,IS,NO,RU)	0.1301	6735					
(EU,FO,IS,NO),(RU)	0.106	5540					2100
(EU,FO,IS,RU),(NO)	0.106	4645				2995	
(EU,FO,NO,RU),(IS)	0.106	5352			2288		
(EU,IS,NO,RU),(FO)	0.106	4929		2711			
(FO,IS,NO,RU),(EU)	0.106	5496	2144				
(EU),(FO),(IS),(NO),(RU)	0.0688		2009	2585	2198	2872	2021
Scenario 1*c*							
(EU,FO,IS,NO,RU)	0.1301	6735					
(EU,FO,IS,NO),(RU)	0.113	5453					1684
(EU,FO,IS,RU),(NO)	0.113	4882				2255	
(EU,FO,NO,RU),(IS)	0.113	4882			2255		
(EU,IS,NO,RU),(FO)	0.113	4882		2255			
(FO,IS,NO,RU),(EU)	0.113	5437	1701				
(EU),(FO),(IS),(NO),(RU)	0.0815		1584	2160	2160	2160	1627
Scenario 2*a*							
(EU,FO,IS,NO),(RU)	0.111	6071					1253
(EU,FO,IS),(NO),(RU)	0.0955	5716				1863	1272
(EU,FO,NO),(IS),(RU)	0.0955	5054			2925		1272
(EU,IS,NO),(FO),(RU)	0.0955	5054		2925			1272
(FO,IS,NO),(EU),(RU)	0.0955	4874	2705				1272
(EU),(FO),(IS),(NO),(RU)	0.0765		2537	2699	2699	1812	1219
Scenario 2*b*							
(EU,FO,IS,NO),(RU)	0.106	6107					1553
(EU,FO,IS),(NO),(RU)	0.0895	5435				2136	1495
(EU,FO,NO),(IS),(RU)	0.0895	4800			2772		1495
(EU,IS,NO),(FO),(RU)	0.0895	4800		2772			1495
(FO,IS,NO),(EU),(RU)	0.0895	4691	2881				1495
(EU),(FO),(IS),(NO),(RU)	0.0688		2734	2706	2706	2093	1446
Scenario 2*c*							
(EU,FO,IS,NO),(RU)	0.106	6107					1553
(EU,FO,IS),(NO),(RU)	0.0895	4907				2665	1495
(EU,FO,NO),(IS),(RU)	0.0895	4907			2665		1495
(EU,IS,NO),(FO),(RU)	0.0895	4907		2665			1495
(FO,IS,NO),(EU),(RU)	0.0895	5006	2566				1495
(EU),(FO),(IS),(NO),(RU)	0.0688		2444				1446

Note: See Table 1 for abbreviations.

$$V_i(X_i, X_{-i}) = \sum_{y=0}^{\infty} \frac{\sum_{j=1}^{6} \Pi_{i,j,y}}{(1+r)^y} \tag{8}$$

where *r* is the rate of discount (a 5% discount rate is used throughout the calculations).

We can now use this (eq. 8) to find the best-response (eq. 4) Nash equilibria (eq. 5) for all coalitions and players in a given coalition structure. Then we can proceed with the analysis of the stability of the coastal state coalition.

Results

The best-response Nash equilibrium (cf. eqs. 4, 5, and 8) for each coalition structure in the game (Tables 7 and 9) are used to calculate the coalition values (Tables 8 and 10) of the coastal state coalition for scenario 1 and scenario 2, respectively, by subtracting the sum of the noncooperative payoffs to the individual coastal states from the payoff to the coastal state coalition (cf. eq. 6). The coastal states individual (coalition) values from unilateral deviation from the

Table 7. Scenario 1 — best-response Nash equilibria (net present values in million NOK).

	Total	CS	EU	FO	IS	NO	RU
Scenario 1*a*							
(EU,FO,IS,NO),(RU)	6934	3635					3299
(EU,FO,IS),(NO),(RU)	5640	2267				1712	1662
(EU,FO,NO),(IS),(RU)	5771	2252			1814		1704
(EU,IS,NO),(FO),(RU)	5771	2252		1814			1704
(FO,IS,NO),(EU),(RU)	5982	2017	2283				1682
(EU),(FO),(IS),(NO),(RU)							
Mean	4886	4055*	1228	961	961	905	831
Maximum			2546	2223	2223	1971	1820
Minimum			0	0	0	0	0
Scenario 1*b*							
(EU,FO,IS,NO),(RU)	6972	3699					3273
(EU,FO,IS),(NO),(RU)	6392	2947				2582	864
(EU,FO,NO),(IS),(RU)	6535	3115			2744		676
(EU,IS,NO),(FO),(RU)	6535	3115		2744			676
(FO,IS,NO),(EU),(RU)	6684	2808	3198				678
(EU),(FO),(IS),(NO),(RU)							
Mean	5124	4121*	1193	1003	1003	922	1003
Maximum			2955	2509	2509	2233	2298
Minimum			0	0	0	0	0
Scenario 1*c*							
(EU,FO,IS,NO),(RU)	6972	3699					3273
(EU,FO,IS),(NO),(RU)	5806	2017				2265	1524
(EU,FO,NO),(IS),(RU)	5806	2017			2265		1524
(EU,IS,NO),(FO),(RU)	5806	2017		2265			1524
(FO,IS,NO),(EU),(RU)	6420	2715	2841				865
(EU),(FO),(IS),(NO),(RU)							
Mean	5128	4120*	1056	1021	1021	1021	1008
Maximum			2494	2435	2435	2435	2357
Minimum			0	0	0	0	0

Note: See Table 1 for abbreviations.

*The sum of NPVs from the coastal states acting as singletons.

Table 8. Scenario 1 — coalition values (in million NOK).

Scenario	CS	EU	FO	IS	NO
1*a*	–420	1055	853	853	807
1*b*	–422	2005	1741	1741	1660
1*c*	–421	1785	1244	1244	1244

Note: See Table 1 for abbreviations.

coastal state coalition while the others remain in the coalition are found by subtracting the individual country's noncooperative payoff from the payoff from unilaterally deviating from the coastal state coalition (Tables 8 and 10).

Scenario 1

In scenario 1, the spawning takes place in the EEZs of the EU, the Faroe Islands, and Iceland, in scenario 1*c* in Norway's EEZ as well, and there is no blue whiting in Russia's EEZ (Table 2). Hence, Russia is not a partner in the blue whiting agreement and therefore always operates as a DWFN.

We see that the benefits provided in terms of coalition values when all of the coastal states cooperate in a coalition are negative (NOK –420 million, –422 million, and –421 million for scenarios 1*a*, 1*b*, and 1*c*, respectively; Table 8). For the coastal state coalition to be stand-alone stable, its value has to be greater than the sum of its members' individual coalition values. This is clearly not the case; the individual coalition values are all positive, whereas the coastal state coalition values are negative (Table 8). Thus, in the scenario in which a warmer climate leads to a more westerly distribution of the blue whiting stock, a coastal state coalition cannot be stable under any circumstances.

Scenario 2

We now continue with the scenario (Table 3) in which the distribution of the blue whiting expands eastward into the Barents Sea such that Russia will become a coastal state and the grand coalition, (EU,FO,IS,NO,RU) and the coastal state coalition are identical (Table 9).

We see that although the grand coalition's values (NOK 3504 million, 2666 million, and 2949 million for scenarios 2*a*, 2*b*, and 2*c*, respectively; Table 10) are all positive, they are not sufficiently large to compensate the coastal states for their individual values of free-riding the coalition while the others remain in the coalition. This is the criterion for a coalition not to be stand-alone stable (cf. the discussion of stand-alone stability under the game theory section). Therefore, in scenario 2, the grand coalition, in a strict sense, cannot be said to be a stable coalition structure.

Table 9. Scenario 2 — best-response Nash equilibria (net present value in million NOK).

	Total	CS	EU	FO	IS	NO	RU
Scenario 2*a*							
(EU,FO,IS,NO,RU)	7871						
(EU,FO,IS,NO),(RU)	7074	3852					3222
(EU,FO,IS,RU),(NO)	7170	3708				3462	
(EU,FO,NO,RU),(IS)	7102	3801			3302		
(EU,IS,NO,RU),(FO)	7481	6079		1402			
(FO,IS,NO,RU),(EU)	7417	5868	1549				
(EU),(FO),(IS),(NO),(RU)							
Mean	4367		1024	903	775	882	784
Maximum			2178	2072	1932	2066	1743
Minimum			0	0	0	0	0
Scenario 2*b*							
(EU,FO,IS,NO,RU)	7871						
(EU,FO,IS,NO),(RU)	7792	1935					5857
(EU,FO,IS,RU),(NO)	6901	3565				3337	
(EU,FO,NO,RU),(IS)	6887	3644			3243		
(EU,IS,NO,RU),(FO)	6934	3507		3427			
(FO,IS,NO,RU),(EU)	6977	3513	3464				
(EU),(FO),(IS),(NO),(RU)							
Mean	5205		1095	1077	1046	1039	947
Maximum			2590	2607	2482	2847	2556
Minimum			0	0	0	0	0
Scenario 2*c*							
(EU,FO,IS,NO,RU)	7871						
(EU,FO,IS,NO),(RU)	6774	3810					2964
(EU,FO,IS,RU),(NO)	6880	3621				3259	
(EU,FO,NO,RU),(IS)	6880	3621			3259		
(EU,IS,NO,RU),(FO)	6880	3621		3259			
(FO,IS,NO,RU),(EU)	6996	3592	3404				
(EU),(FO),(IS),(NO),(RU)							
Mean	4922		1068	1019	1019	1019	797
Maximum			2431	2335	2335	2335	2056
Minimum			0	0	0	0	0

Note: See Table 1 for abbreviations.

Table 10. Scenario 2 — coalition values (in million NOK).

Scenario	CS	EU	FO	IS	NO	RU
2*a*	3504	525	499	2527	2580	2438
2*b*	2666	2369	2350	2197	2298	4910
2*c*	2949	2336	2240	2240	2240	2167

Note: See Table 1 for abbreviations.

Let us now consider the stability of the coastal state coalition if unilateral deviation is not an option, but any deviation from the coastal state agreement breaks down any coalition, and all the players revert to noncooperative behaviour. As shown for scenario 2 (Table 7), there is no unique solution when all act as singletons. There are multiple strategy combinations that can be considered best response for all players. The maximum solutions are probably not feasible for all players simultaneously, because this would mean that all nations would have to fish with such high effort that the stock would collapse, and the minimum is zero for all players. However, if the average (mean) NPVs (Table 7) can be taken as an example of what the players can expect to gain by acting noncooperatively, the sum of all of the singleton NPVs is less than the NPV to the grand coalition. The sum of the NPVs of the coastal states when they all act noncooperatively (NOK 4367 million, 5205 million, and 4922 million for scenarios 2*a*, 2*b*, and 2*c*, respectively) are less than NOK 7871 million (Table 7), the NPV of the grand coalition. Thus, the coastal state agreement can be considered stable and the Nash equilibrium of the coalition game.

Note that this does not apply under scenario 1. Recall that coastal state coalition values were all negative (Table 8), and the formation of a coastal state coalition would not be rational.

It is important to note that in the presence of non-unique equilibrium, these results were based on the average of all the different possible solutions. If we had chosen one of the possible solutions, the cooperative solution could possibly be a better solution than the sum of the singleton's NPVs of the coastal states. However, because of the lack of a better equilibrium selection criteria, in the presence of multiple equilibria we decided use the average of the equilibria NPVs as a representation of the NPVs that the players could expect in the coalition structure where nonuniqueness occurs.

Discussion

This paper analysed how different blue whiting distribution scenarios might affect the formation, stability, and success of the coastal state coalition on the management of the Northeast Atlantic blue whiting fish stock. We assume that the blue whiting will change its migration pattern and distribution area in response to changes in the ocean environment. Two possible distributional scenarios were analyzed. In the first scenario, the blue whiting is distributed in a more westerly direction, abandoning Russian waters altogether. In the second scenario, an increased sea temperature in the Norwegian Sea and the Barents Sea shifts the distribution of the blue whiting in a northeasterly direction with spawning activity in Norwegian waters and blue whiting catches in Russian waters, making Russia a member of the coastal state coalition.

Based on these scenarios, we formulated two possible combinations of quarterly shares. Each share represents the fraction of the stock available for harvest in a certain area, i.e., the different exclusive economic zones or international waters, at certain times. These shares, along with the bioeconomic model, were used to calculate the best-response Nash equilibria, based on the NPVs, to coalitions and players under different coalition structures.

Finally, this allowed us to analyse the coalition formation, success, and stability in particular coalitions among the coastal states. The coalition analysis indicates that the stability of the blue whiting agreement between the coastal states would remain unchanged relative to today's agreement (cf. Ekerhovd (2008)) if global warming means an increase in sea temperatures in the Norwegian Sea and the Barents Sea. However, if the distribution remains as it is today and the stock is shared among the EEZs of the EU, the Faroe Islands, Iceland, and Norway and the high seas areas in the Northeast Atlantic, excluding Russia as a coastal state, this would weaken the stability of the current coastal state agreement on the management of the blue whiting stock.

In scenario 2, with increased ocean temperatures and salinity in the Norwegian Sea and the Barents Sea, we assumed that the blue whiting migrated into Russian waters and that Russia achieved the status of being a coastal state with regard to the management of this stock. The change in status from being regarded as a DWFN by the original coastal states to being accepted and included as a coastal state in the management of a straddling fish stock when the stock for some reason changes its migration pattern and distribution is not necessarily a straightforward process. It might take years before the new status is generally accepted by the others, as the shift in the distribution can be a gradual process with a considerable amount of short-term variation, meaning that there may be considerable doubt as to whether a shift in distribution is only a temporary change or if the fish stock actually has changed its migration pattern and area of distribution permanently. During the period of transition, the underlying uncertainty might put an established agreement on the management of the stock among the original coastal states at risk, as the emerging coastal state tries to prove its claim to the stock by severely increasing its fishing effort and thus its catches to establish rights to the fishery and gain acceptance for its new status. The original coastal states members might try to limit the prospective coastal state's profit by increasing their fishing efforts too. If this transient period lasts for a long time and the noncooperative behaviour is allowed to continue, it might threaten the fishery, as the stock cannot sustain a too high fishing mortality indefinitely without either becoming extinct or being driven to the break-even stock level (the level at which further fishing becomes unprofitable).

However, when an agreement that includes all countries is finally reached, as in the case of scenario 2, the coastal state coalition will act as a sole owner and not as in scenario 1, where Russia always acts as a singleton player while the coastal state coalition maximizes its own profit, taking the action of Russia as given. With the sole-owner NPV being the maximum attainable value, the players in scenario 2 will never find themselves in a situation like scenario 1, where the sum of the NPVs in a coalition structure where some or all players act as singletons exceeds the NPV to the coastal state coalition.

In scenario 1, Russia is no longer regarded as a coastal state; the coalition of coastal states is no longer stable, even if the coalition formation options were restricted to full cooperation among the coastal states or reversion to a state where all act as singletons. In the opposite case, the coastal state coalition would be stable if such a restriction were put on the coalition structure. However, if this is not the case, the individual members of the coastal state coalition would have incentives to free ride on the agreement if the remaining coalition continued to cooperate. What has become evident from our exercise is that if the distribution area of the blue whiting stock in the Northeast Atlantic were to be reduced, the cooperation among the coastal states would become even more difficult than it is already.

In the second climate change scenario in which the Norwegian and Barents seas are expected to warm up and the distribution of the blue whiting stock is expected to expand northeastward into the EEZ of Russia, the coastal state coalition would be stable if the option of the member states to free ride on the agreement for some reason did not exist. Then the NPV of the coastal state coalition would always exceed the sum of NPVs to the coastal states acting as singletons, and the coastal states would be better off cooperating in a coalition. However, when the coastal state coalition does not include all of the countries that participate in the fishery, as is the case in the first scenario and in Ekerhovd (2008), Russia is excluded from participating in the coastal state coalition, the coalition NPV is less than a potential grand coalition NPV would be, and a mechanism that prohibits free-riding among the coastal states is not necessarily sufficient to make the coastal states coalition stable. An example where this turns out to be true is scenario 1 of this paper. What might help remedy this weakness is for the coastal states to transfer some of their sovereignty over the fish stock staying in their national EEZs to a regional fisheries management organization (RFMO) and let it manage the fish stock. According to the law of the sea, membership in a RFMO is open to all countries with real interest in the fish stock (Bjørndal and Munro 2003). The open membership of the RFMO guarantees a share of the profits to all interested parties, as well as being able to provide a higher NPV than any partial cooperation. Furthermore, if it is able to enforce mechanisms that will deter its members from free-riding, the prospects for cooperation will be improved.

However, it is possible that this is partially achieved in the management of the blue whiting stock. The coastal states agree on a total allowable catch (TAC) for the stock. This TAC is then divided among coastal states, and in addition, a share thereof is set aside to be harvested in international waters. The local RFMO, the North East Atlantic Fisheries Commission (NEAFC), is given the responsibility of dividing this share among all interested parties, including Russia. Moreover, Russia could be further accommodated by exchange of quota in their waters against being allowed to fish some of the coastal states' shares in their respective EEZs. This can be seen as a way of sharing the benefits of cooperation through side payments, and by providing higher benefit than a simple coastal state regime would be able to, a more stable management is achieved.

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Appendix A. Management plan for the blue whiting fishery

In December 2005, the coastal states (EU, Norway, Iceland, and Faroe Islands) agreed on a sharing arrangement for the blue whiting stock (Agreed record of conclusion of fisheries consultations on the management of the blue whiting stock in the north-east Atlantic. An agreement between the blue whiting coastal states consisting of the EU, Denmark, on behalf of the Faroe Islands, Iceland, and Norway, December 2005; Anonymous 2006). This arrangement provides for catches in 2006 of 2 million tonnes, allocated as follows: EU, 30.5%; Faroe Islands, 26.125%; Norway, 25.745%; and Iceland, 17.63%. Russia will be accommodated by transfers from some of the coastal states and additional catches in the NEAFC regulatory area (International Council for the Exploration of the Sea 2006).

Agreed Record of Conclusions of Fisheries Consultations Between the Faroe Islands, the European Community, Iceland and Norway on the Management of the Blue Whiting Stock in the North-East Atlantic in 2006

1. A Delegation of the European Community, the Faroe Islands, Iceland and Norway met in Oslo on 15 and 16 December 2005 to consult on the management of the Blue Whiting stock in the North-East Atlantic.
2. The Delegations agreed to recommend to their respective authorities the arrangement for the regulation of the fisheries of Blue Whiting in 2006 and subsequent years set out in Annex I to this Agreed Record. They also agreed to recommend to their respective authorities the multi-annual management arrangement set out in Annex II.
3. The Delegations agreed to recommend that, in 2006, ICES be requested to evaluate, as soon as possible, whether the multi-annual management arrangement as

set out in Annex II is in accordance with the precautionary approach and to make the results of this evaluation available to the Parties. The Delegations agreed to review the multiannual management arrangement on the basis of evaluation by ICES.

4. This Agreed Record, including bilateral arrangements related to the implementation thereof, shall be applied provisionally from 1 January 2006 and enter into force when all Parties have notified each other of the completion of their necessary procedures.
5. For subsequent years, Delegations agreed to allocate allowable catches in the proportions that are set out in paragraph 1 of Annex I.
6. Unless one or more of the Parties notifies its withdrawal not later than by the end of June, the Agreed Record shall be renewed annually, including Annexes, in which years, maximum catch limit and quotas are updated.
7. The Delegations agreed to inform the NEAFC Secretariat about the regulatory measures they intend to take on the basis of this Agreed Record, for the fisheries of Blue Whiting in 2006 and in subsequent years.

Annex I. Arrangement for the regulation of the fisheries of Blue Whiting in 2006

1. In accordance with the multi-annual management arrangement for the fisheries of Blue Whiting set out in Annex II to this Agreed Record, the Parties agree to restrict their fisheries of Blue Whiting in 2006 to a maximum catch limit of 2,000,000 tonnes on the basis of the following quotas:
    - European Community 610 000 tonnes
    - Faroe Islands 522 500 tonnes
    - Iceland 352 600 tonnes
    - Norway 514 900 tonnes
2. Each Party may transfer unutilized quantities of up to 10% of the quota allocated to it for 2006 to 2007. Such transfer shall be in addition to the quota allocated to the Party concerned for 2007.
3. In the event of overfishing of the allocated quotas by any Party in 2006, the quantity shall be deducted from the quota allocated in 2007 for the Party or Parties concerned.
4. The Parties may fish Blue Whiting within the quotas laid down in paragraph 1 in their respective zones of fisheries jurisdiction and in international waters.
5. Further arrangements by the Parties, including arrangements for access, quota transfers and other conditions for fishing in the respective zones of fisheries jurisdiction, are regulated by bilateral arrangements.

Annex II. Arrangement for the multi-annual management of the Blue Whiting stock

1. The Parties agree to implement a multi-annual management arrangement for the fisheries on the Blue Whiting stock which is consistent with the precautionary approach, aiming at constraining harvest within safe biological limits, protecting juveniles, and designed to provide for sustainable fisheries and a greater potential yield, in accordance with advice from ICES.
2. The management targets are to maintain the Spawning Stock Biomass (SSB) of the Blue Whiting stock at levels above 1.5 million tonnes ($B_{\lim}$) and the fishing mortality rates at levels of no more than 0.32 (F_{pa}) for appropriate age groups as defined by ICES.
3. For 2006, the Parties agree to limit their fisheries of Blue Whiting to a total allowable catch of no more than 2 million tonnes.
4. The Parties recognise that a total outtake by the Parties of 2 million tonnes in 2006 will result in a fishing mortality rate above the target level as defined in paragraph 2. Until the fishing mortality has reached a level of no more than 0.32, the Parties agree to reduce their total allowable catch of Blue Whiting by at least 100,000 tonnes annually.
5. When the target fishing mortality rate has been reached, the Parties shall limit their allowable catches to levels consistent with a fishing mortality rate of no more than 0.32 for appropriate age groups as defined by ICES.
6. Should the SSB fall below a reference point of 2.25 million tonnes (B_{pa}), either the fishing mortality rate referred to in paragraph 5 or the tonnage referred to in paragraph 4 shall be adapted in the light of scientific estimates of the conditions then prevailing. Such adaptation shall ensure a safe and rapid recovery of the SSB to a level in excess of 2.25 million tonnes.
7. This multi-annual management arrangement shall be reviewed by the Parties on the basis of ICES advice.

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Appendix B. NEAFC regulatory measurements

In addition to the coastal states management plan, the following regulatory measures for the blue whiting stock for 2006 are suggested by NEAFC (International Council for the Exploration of the Sea 2006).

1. NEAFC takes note of the Agreed Record of Conclusions of Fisheries Consultations on the Management of the Blue Whiting Stock in the North-East Atlantic for 2006 between the European Community, the Faroe Islands, Iceland and Norway signed in Oslo on 16 December 2005.
2. NEAFC further notes that by way of the said Agreed Record, the aforementioned Parties agreed to restrict their fishery on the Blue Whiting Stock in 2006 on the basis of specified quotas according to a total catch limitation of 2 million tonnes.
3. In accordance with Article 5 of the Convention on Future Multilateral Cooperation in North-East Atlantic fisheries,

the Contracting Parties recommend the following measure for the Blue Whiting Stock for 2006.

(*a*) To ensure consistency and compatibility with the said Agreed Record, NEAFC hereby establishes an allowable catch limitation of 253 000 tonnes of Blue Whiting for 2006 in waters beyond the areas under national fisheries jurisdiction of the Contracting Parties

(*b*) This allowable catch limitation shall be allocated as follows:

- European Community 44 000 tonnes (*)
- Norway 37 000 tonnes (*)
- Denmark in respect of:
- Faroe Islands 37 000 tonnes (*)
- Greenland 10 000 tonnes
- Iceland 25 000 tonnes (*)
- Russian Federation 100 000 tonnes

4. The quotas referred to in paragraph 2 may be fished in the areas defined in paragraph 3*a*.

(*) Catches taken under these allocations shall be deducted from quotas allocated to Parties to the said Agreed Record.

Reference

International Council for the Exploration of the Sea. 2006. Report of the Northern Pelagic and Blue Whiting Working Group. ICES CM 2006/ACFM:34.

Forecasting the distribution of the invasive round goby (*Neogobius melanostomus*) in Wisconsin tributaries to Lake Michigan

Matthew S. Kornis and M. Jake Vander Zanden

Abstract: The Laurentian Great Lakes host more than 180 non-native species, including several that have resulted in major economic and ecological effects. This list includes the round goby (*Neogobius melanostomus*), an aggressive, benthic Ponto-Caspian fish that has established large populations in coastal Great Lakes habitats. Here, we document the inland dispersal of round gobies into Wisconsin tributaries of Lake Michigan. Round gobies were detected in 26 of 73 streams (36%) and found >10 km upstream of Lake Michigan in nine watersheds. Round goby presence–absence was modeled using landscape-scale data from these invaded streams. We forecasted the future spread of round goby within Wisconsin's Lake Michigan basin using our best model (80% accuracy), which included watershed area, stream gradient, and watershed slope as predictors. Round gobies were predicted to invade 1369 km of stream habitat up to the first stream barrier, and 8878 km of stream was identified as suitable looking beyond barriers at the broader Lake Michigan watershed (Wisconsin only). Our results depict the Great Lakes as a springboard for invasive species to disperse into inland ecosystems and, because round gobies are not usually reported in small streams in their native range, emphasize the utility of data from invaded regions when forecasting invasive species distributions.

Résumé : Les Grands Lacs Laurentiens contiennent >180 espèces non indigènes dont plusieurs ont eu des effets économiques et écologiques importants. Cette liste comprend le gobie à taches noires (*Neogobius melanostomus*), un poisson agressif d'origine ponto-caspienne, qui a formé de grandes populations dans les habitats côtiers des Grands Lacs. Nous apportons des informations sur la dispersion vers l'intérieur des gobies à taches noires dans les tributaires du lac Michigan au Wisconsin. Les gobies à taches noires se retrouvent dans 26 de 73 cours d'eau (36 %) et à >10 km en amont du lac Michigan dans neuf bassins versants. Nous avons modélisé la présence–absence des gobies à taches noires à l'aide de données à l'échelle du paysage provenant des cours d'eau envahis. Nous prédisons la dispersion future du gobie à taches noires dans le bassin versant du lac Michigan au Wisconsin à l'aide de notre meilleur (80 % d'exactitude) modèle qui inclut la surface du bassin versant, le gradient du cours d'eau et la pente du bassin comme variables prédictives. Notre prédiction est que les gobies à taches noires vont envahir 1369 km d'habitat lotique jusqu'à la première barrière dans les cours d'eau; de plus, 8878 km de cours d'eau au-delà des barrières paraissent des habitats convenables dans le bassin élargi du lac Michigan (dans le seul Wisconsin). Nos résultats décrivent les Grands Lacs comme des tremplins pour les espèces envahissantes vers les écosystèmes de l'intérieur; comme les gobies à taches noires ne se retrouvent pas généralement dans les petits cours d'eau dans leur aire de répartition indigène, nos résultats soulignent l'utilité de données provenant des régions envahies pour la prédiction des répartitions des espèces envahissantes.

[Traduit par la Rédaction]

Introduction

Increases in global transport and trade have resulted in an accelerating pace of species invasions at substantial ecological and economic costs (Mack et al. 2000; Pimentel et al. 2005). Understanding the nature and consequences of species invasions continues to be an urgent challenge facing the scientific community (Kolar and Lodge 2001; Sakai et al. 2001). Historically, invasive species studies have focused on documenting the human-facilitated movement of species and their effects on ecosystems and native biota. The realization that most successful invasions are irreversible has motivated ecologists to attempt to forecast species movements before populations become irreversibly entrenched in new ecosystems (Ricciardi and Rasmussen 1998; Vander Zanden 2005). This problem has been approached by evaluating the invasive potential of species not yet observed in a given ecosystem (Ricciardi and Rasmussen 1998; Kolar and

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Lodge 2002) and by modeling the dispersal of species that have already invaded neighboring ecosystems (Leung et al. 2006; Keller et al. 2008). Despite such efforts, producing quantitative predications about invader success continues to challenge the scientific community (Vander Zanden 2005; Lodge et al. 2006; Hastwell et al. 2008).

One challenge in forecasting the spread of invasive species is acquiring appropriate data on which to base predictions. Forecasting invader distributions is commonly done using ecological niche modeling, which assumes that species will be able to invade areas that have the same ecological characteristics as their native range (Peterson 2003; Mercado-Silva et al. 2006). Yet species populations often behave differently in invaded ecosystems due to a release from certain predators, competitors, or parasites (Keane and Crawley 2002; Blumenthal 2006). In some cases, models based on data from regions already invaded by a given species (hereafter called invaded range data) have been found to be more accurate at forecasting that species' distribution than data from its native range (Loo et al. 2007). The greater utility of invaded range data is likely due to the fact that species may act unpredictably outside of their native range by adapting to novel habitats (Mooney and Cleland 2001; Peterson and Vieglais 2001).

The prevalence of round gobies (*Neogobius melanostomus*) in the Great Lakes region provides an alternative to using habitat data from the species' native range in developing models to predict species spread. The round goby is a small benthic fish (2–20 cm) native to the Black, Caspian, and Azov seas and the lower reaches of large associated rivers (Charlebois et al. 1997). The species was first observed in the Great Lakes in 1990, has since spread to all five Great Lakes, and is still expanding in the region (Jude et al. 1992; Jude 2001). Despite being limited to large lakes and rivers in their native range, round gobies have been found in four small (drainage area of 30–140 km^2) tributaries to Lake Erie (Phillips et al. 2003; Donald Luttman, Pennsylvania Department of Environmental Protection, 230 Chestnut Street, Meadville, PA 16335, USA, personal communication, 21 January 2009), illustrating the potential for round gobies to invade small lotic systems. Though the round goby was likely transported to the Great Lakes via ballast water in large freight ships (Hensler and Jude 2007), the species has access to the extensive network of Great Lakes tributaries independent of human assistance. In such cases, the range of a species expands as a result of population growth and natural dispersal such that vulnerable ecosystems can be identified by coupling species life history information with habitat suitability data (Vander Zanden et al. 2004). Although high predator abundance could slow or limit round goby expansion, physical barriers such as dams and waterfalls will ultimately limit upstream migration, and habitat suitability will likely dictate the range of round goby in inland streams up to the first stream impediment.

Accurately predicting the spread of round goby to inland streams is of great interest because of the detrimental effects that this species has wrought on the ecosystem of the Laurentian Great Lakes. Round gobies feed voraciously on benthic invertebrates and fish eggs (Diggins et al. 2002; Carman et al. 2006) and prefer rock or cobble substrate for spawning and feeding habitat (Jude et al. 1995; Charlebois et al. 1997; Corkum et al. 2004). Round gobies outcompete native benthivorous fish for feeding and spawning habitat because they are generally larger and more aggressive (Dubs and Corkum 1996; Janssen and Jude 2001; Balshine et al. 2005). Round gobies threaten to deplete food sources and extirpate native species in part because of their capacity to reach large population densities quickly, with females spawning four to five times per season and male nest-guarding resulting in a 95% egg hatching rate (MacInnis and Corkum 2000). Round gobies also provide a pathway for transferring contaminants such as mercury and polychlorinated biphenyls (PCBs) from *Dreissenid* mussels to piscivorous fishes potentially consumed by humans (Hogan et al. 2007; Ng et al. 2008).

Here we document the distribution of round goby in Wisconsin tributaries to Lake Michigan. We use habitat data (both field-based and landscape-scale) from the round goby's invaded range to model the distribution of the species in Wisconsin's Lake Michigan tributaries. Using this model, we then forecast the potential distribution of round gobies in the Lake Michigan watershed, both with and without dispersal barriers.

Materials and methods

Site selection

We conducted a census of all Wisconsin tributaries of Lake Michigan for round goby presence–absence from 9 June to 7 August 2007 (130 sites on 73 distinct streams; see Supplemental Table S1 for coordinates, available online from NRC Data Depository[2]). We defined a tributary as continuous flowing water that interfaced with Lake Michigan. Streams ranged in size from less than 1 m to over 600 m wide and provided a diverse range of habitat attributes. Sites were selected based on accessibility, with sampling occurring most often at bridge crossings, public parks, and, less frequently, on privately owned land. For a given stream, we sampled at or near the mouth of the stream first and continued to sample at sites upstream until no round gobies were detected. We assumed that if round gobies were not present at a given site, they would be absent upstream of that site as well. This was done to minimize the potential effect of an unsaturated landscape on our forecast of round goby distribution. We chose to maximize the geographic area covered by our study, and thus the number of systems examined, by sampling discrete sites (minimum distance of 1 stream km between sample sites from the same stream).

Fish collection

Two collection methods were required to adequately sample the wide range of stream sizes examined in this study. Sites that were wadeable (<1 m in depth) and exhibited high water clarity were sampled using a battery-powered backpack electrofishing unit with pulsed DC current. Rate (electric pulse frequency) and duty (pulse width divided by pulse period) were set to 80 and 90, respectively, to target smaller fish. Because of the variability in conductance between sites, we manipulated voltage settings by site to maintain a current between 3.5 and 4 amps. Rather than electrofishing a set area, we sampled for a set amount of time (20 min) at each site, working upstream and sampling from bank to bank.

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[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5348. For more information on obtaining material, refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

We could not electrofish murky waters because round gobies lack a swim bladder and remain on the bottom when electrofished, making them difficult to see in dark water. Therefore, sites that were too turbid or deep (>1 m in depth) to electrofish were sampled using an overnight set of 10 cylindrical, wire-mesh, Gee-style minnow traps baited with chicken liver. Minnow traps had a mesh size of 6 mm with openings that were 30 mm in diameter. Traps were set in or near cobble or boulder substrate if available to maximize the likelihood that the traps would detect round goby presence. Traps were deployed for a minimum of 16 h overnight and were collected the following day.

Both of these capture methods have been recommended for use in capturing round gobies (Phillips et al. 2003; Diana et al. 2006). Probability of detection for both methods was assumed to be 1 in areas of high round goby abundance. Probability of detection at sites where round gobies were rare was 0.75 for electrofishing as determined by resampling sites where we had previously only captured one round goby and calculating the percentage of resamples in which we detected round gobies ($n = 12$). Probability of detection using minnow traps was not calculated but was assumed to be at least as high as the electrofishing method, as evidenced by the high percentage of sites where round goby presence was detected using this method (round gobies were detected at 57% of trapped sites compared with 40% of electrofished sites) and the high number of round gobies usually captured by minnow traps (geometric mean of 9.7 round gobies per set of 10 minnow traps at sites where round gobies were detected (95% confidence interval 0.9–108.1)).

Round gobies were euthanized using tricaine methanesulfonate (MS-222) and preserved in 95% ethanol. All other fish species were counted and released at the site of capture.

Habitat data

We collected a suite of local habitat characteristics specific to each site sampled (hereafter called field-based data). Water temperature and conductivity were collected using an YSI 85 meter (YSI Inc., Yellow Springs, Ohio). Bankfull width was measured using a Bushnell laser rangefinder (Bushnell Corp., Overland Park, Kansas). Stream depth was measured at 1/4, 1/2, and 3/4 of bankfull width, and values were averaged for each site. Percent substrate composition was visually assessed as silt, sand, gravel, cobble, boulder, or bedrock. In turbid and deep streams, substrate was assessed tactilely by wading throughout the site. Global positioning system (GPS) coordinates of each site were recorded using a Garmin GPSMap 76S unit (Garmin Ltd., Olathe, Kansas), and stream distance from Lake Michigan was determined for each site using a geographic information system (GIS).

Landscape-scale variables (watershed size, land use (percentage as urban, agriculture, forest, wetland), stream gradient, watershed slope, soil permeability, base-flow index (90% exceedance flow/watershed area), and mean July air temperature) and the coordinates of Wisconsin dams were obtained from a GIS database (US Geological Survey, Great Lakes Aquatic GAP Analysis Project). Landscape-scale variables were calculated on the scale of confluence-bound stream segments (each segment between the confluence of two streams was considered one unit).

Data analysis

Empirical round goby presence–absence data were mapped using a GIS, and landscape-scale variables associated with each site were determined. A correlation analysis was performed on all habitat variables, and those that were highly correlated with several other parameters were discarded ($r^2 > 0.6$ with two or more other covariates). The candidate set of predictors was composed of the remaining independent variables (Table 1). Two variables, watershed area and bankfull width, were log-normally distributed and were log-transformed to produce a nonskewed distribution. Nonskewed variables are not an assumption of generalized linear mixed models, but there is concern that comparing skewed variables with a set of relatively nonskewed variables would result in bias. Each variable was z-standardized using the equation

$$z = (x - \bar{x})/s$$

where z is the standardized value, x is the observed value, $\bar{x}$ is the mean of all observed values, and s is the standard deviation of all observed values. This converted all variables to the same unitless scale, allowing comparison of the relative effect of each predictor on round goby presence–absence.

Eleven sites that were upstream of a dam (none of which had round gobies present) were removed from the analysis because the dam likely prohibited dispersal. A generalized linear mixed model was used to evaluate which habitat factors were the best predictors of round goby presence–absence at the remaining 119 sites. We used a mixed-effects model in which stream was represented as a random effect to account for correlation between sites from the same stream. The general form of this model is

$$y = \operatorname{logit} P = \beta_0 + \beta_n X_n + \rho_i$$

where β_0 is the intercept, β_n and X_n are the effects of the covariates, and ρ_i is the stream-specific random effect defined as $\rho_i \sim \mathrm{N}(0, \sigma^2)$. Probability of round goby presence can be calculated by

$$P(\text{goby presence}) = e^y/(1 + e^y)$$

We used model performance on independent data as our means of selecting the model used to make predictions about round goby distribution. Best subsets analysis (see below) was used to narrow the number of potential models to the 10 best models at each spatial scale (landscape-scale data only and landscape-scale data plus site-specific, field-based measurements). This distinction was made because landscape-scale data are more readily available for Wiscon-

Table 1. Description of potential covariates of round goby (*Neogobius melanostomus*) presence–absence in Wisconsin tributaries to Lake Michigan.

Habitat variables	Scale of measurement	Goby present (mean ± 1 SD)	Goby absent (mean ± 1 SD)	Significant $P < 0.05$
Conductivity (mS)	Field based	569.2±170.0	672.5±265.0	Yes
Soft substrate (%)	Field based	32.4±34.6	40.5±35.6	No
Hard substrate (%)	Field based	37.3±29.4	36.5±25.8	No
Bankfull width (m)	Field based	51.7±101.5	15.8±26.5	Yes
Water temperature (°C)	Field based	23.9±3.5	21.7±3.5	Yes
Distance upstream (m)	Field based	5829±5712	2982±5304	Yes
Reach gradient (vertical drop/reach length)	Landscape	0.0023±0.0057	0.0045±0.0039	Yes
Watershed slope	Landscape	0.88±0.39	0.83±0.37	No
Watershed area (km^2)	Landscape	1290±3304	223.6±570	Yes
Agricultural land (%)	Landscape	58.1±22.7	53.5±24.2	No
Urban land (%)	Landscape	2.4±6.1	6.4±12.1	Yes
Forested land (%)	Landscape	17.5±15.8	12.9±12.5	No
Wetland (%)	Landscape	13.5±7.8	15.9±15.5	No
Open water (%)	Landscape	0.87±1.6	0.57±2.3	No
Course-grained surficial geology (%)	Landscape	11.4±13.5	4.6±11.3	Yes

Note: Two-tailed *t* tests assuming unequal variance were conducted to detect significant differences between sites with and without round goby present. Distance upstream refers to distance from Lake Michigan "as the fish swims". $n = 66$ absent and 53 present. SD, standard deviation.

sin streams than field-based data, which are limited to a small subset of streams and would therefore limit the utility of a predictive model that required field-based data. Field-based data were included in 10 of the 20 models validated to determine how much predictive ability would be lost if field-based data were excluded. Best subsets analysis fits regression models for all possible combinations of predictor variables up to a set parameter limit and compares these models based on some model comparison criterion (Hosmer et al. 1989). Models were selected using the Bayesian information criterion (BIC) to select among models based on predictive ability. We chose to use BIC as a selection criterion because it selects for simpler models by penalizing the addition of a parameter to a greater degree than other commonly used selection criteria such as Akaike's information criterion (AIC). We wanted to select for more conservative, simple models because such models would likely generate more accurate predictions on novel data.

Each of our 20 "best" models was validated using a sevenfold cross validation in which six sets were combined to train each model and one set was used to test each model. This was repeated seven times such that each fold was the test set one time. This method of data portioning, known as *k*-fold cross validation, was recommended for models predicting presence–absence by Fielding and Bell (1997). We chose to perform a sevenfold cross validation because it resulted in subsets with an equal number of observations (17). For every validation, we established the optimal probability threshold using a receiver operating characteristic (ROC) analysis. ROC curves plot the percentage of correctly predicted presence (sensitivity) vs. the percentage of correctly predicted absence (specificity) over a range of probability thresholds distinguishing between predicted presence and predicted absence (Hosmer and Lemeshow 2000). ROC analysis has been used to evaluate logistic regression models using habitat data as predictors and is often used to determine the optimal probability (p_{opt}) that maximizes the overall percentage of correct predictions (Bonn and Schroder 2001; Jensen et al. 2005). We used the minimized difference threshold (MDT) criterion, which selected p_{opt} as the probability threshold that minimized the difference between sensitivity and specificity. The MDT criterion for selecting a probability threshold has previously been shown to produce more accurate predictions compared with other techniques (Jiménez-Valverde and Lobo 2007).

We used Cohen's kappa as the primary statistic of model performance, though we also report correct classification percentage because of its interpretability. Cohen's kappa is the proportion of agreement between actual and predicted data and is derived from a confusion matrix using the following equation:

$$\kappa = \frac{(a+d) - [(a+c)(a+b) + (b+d)(c+d)]/n}{n - [(a+c)(a+b) + (b+d)(c+d)]/n}$$

where a is a correct presence, b is a false presence, c is a false absence, d is a correct absence, and n is the number of observations. Validation, a step too often skipped in predicting fish species distribution, is needed to determine how robust models generalize to novel data (Olden et al. 2002). Cohen's kappa provides a measure of how often models correctly predict presence or absence after accounting for chance effects (Manel et al. 2001) and is considered a good measure of model predictability unless one case (presence or absence) far exceeds the other (Fielding and Bell 1997). This is not a problem with our data, as round gobies were present at 54 and absent from 65 sites (45% and 55%, respectively). Kappa values are considered to reflect model performance on the following scale: 0–0.4, poor to fair; 0.4–0.6, moderate; 0.6–0.8, excellent; and 0.8–1.0, almost perfect (Landis and Koch 1977).

A GIS was used to map the predicted presence–absence of round goby in Wisconsin streams both downstream of known dams and for the entire Lake Michigan basin. Predictions of round goby presence–absence were made on confluence-bound stream segments, the finest scale available in our GIS database. The landscape-scale-only model was used to generate these predictions because field-based variables were not available for the majority of stream segments in the state. All available data were used to construct the classification model of round goby distribution as recommended by Rencher (1995).

Generalized linear mixed models, best subsets analysis, correlation analysis, and *t* tests were performed using the R statistical package (version 2.7.0; www.r-project.org/). All mapping components of this project were done using ArcGIS software (ESRI, Redlands, California). ROC analysis and model validation were completed in Microsoft Excel®.

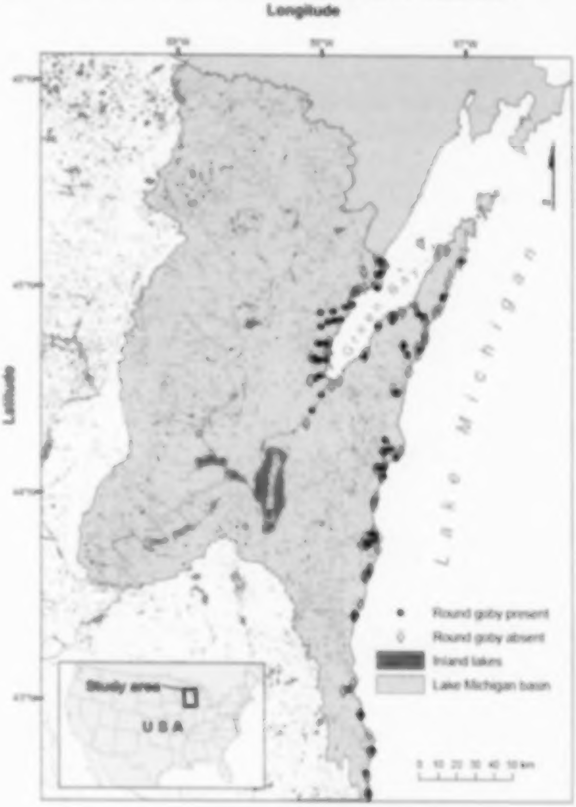
Results

We detected round goby presence in 26 of the 73 sampled watersheds (36%) and 54 of 119 sites (45%; Fig. 1). Round gobies were detected in nine streams at distances greater than 10 km upstream of Lake Michigan, with the farthest inland point recorded at 33.9 stream km in the Pensaukee River (Table 2). We also found round gobies immediately downstream of a physical barrier (dam or waterfall) at seven of the 26 streams (27%) in which round gobies were detected. We sampled upstream of several dams to test whether they served as impediments to round goby migration; no round gobies were detected at above-dam sites (n = 11). Spawning round gobies were captured at several upstream sites, and eggs were found 10.5 km upstream in one watershed. Habitat predictors were evaluated individually for effects on round goby occurrence, and significant differences between sites with and without round gobies were identified for four site-scale variables and four landscape-scale variables (Table 1). According to this analysis, round goby presence had positive relationships with bankfull width, water temperature, watershed area, and the percentage of surficial geology that was coarse-grained. Round goby presence had negative relationships with conductivity, stream gradient, and percentage of urban land.

Validated candidate models were evaluated by their performance on independent data using the mean Cohen's kappa value over the seven validation trials (Supplemental Table S2[2]). On average, models that contained only landscape-scale variables were not significantly different than models that contained both landscape-scale and field-based data (p = 0.76, n = 20, Mann–Whitney U test). Though the best model included both landscape-scale and field-based data (κ = 0.57), it did not perform significantly better on independent data than the best model including only landscape-scale data (κ = 0.55, p = 0.77, n = 7, Student's *t* test assuming equal variance).

We used the best landscape-scale model to forecast the distribution of round goby in Wisconsin tributaries of Lake Michigan. This model displayed moderate agreement between predicted and actual values and correctly classified independent sites 80% of the time. Coefficients in the model were analyzed to determine how the probability of round goby presence changed in relation to each of the variables included in the model. Round goby presence increased linearly with watershed area, decreased linearly with watershed slope, and decreased asymptotically with stream gradient (see Supplemental Table S2[2] for model coefficients). Because dams and waterfalls are barriers to round goby movement, we applied our model to the 3223 km of stream contiguously connected to Lake Michigan (i.e., downstream of any barriers to fish dispersal; Fig. 2*a*). Of these accessible stream reaches, 42% were identified as suitable habitat (1369 km, representing 7% of all Wisconsin streams in the Lake Michigan basin) and may presumably be invaded by round gobies without human assistance. Dam removals or human-assisted movement over dams present the possibility of round gobies circumventing physical barriers. We therefore expanded our forecast to all 20 172 km of Wisconsin streams in the Lake Michigan basin (Fig. 2*b*). Of these stream segments, 44% (8878 km) are predicted to be suitable for round gobies should they gain access to these waters.

Fig. 1. Round goby (*Neogobius melanostomus*) presence (solid circles) and absence (open diamonds) in Wisconsin tributaries of Lake Michigan. Sampling occurred from June to August 2007. Either electrofishing or minnow traps were used depending on the site. Watersheds were sampled at discrete sites upstream from the coast until absence was detected. n = 130 sites and 73 watersheds.



Discussion

Results presented here represent the most extensive documentation of round goby migration into tributary streams of all sizes. Round gobies are native to the Black, Caspian, and

Table 2. Farthest upstream occurrence of round goby (*Neogobius melanostomus*) in invaded Lake Michigan tributaries.

Stream name	Distance upstream (km)
Pensaukee River	33.9
Oconto River	23.2*
Fox River	19.5
Little Suamico River	17.6
East Twin	16.6*
Suamico River	13.3*
Ahnapee River	12.6*
Kewaunee River	11.4*
Sheboygan River	10.5
West Twin	9.6*
Duck Creek	6.4
Milwaukee River	5.5
Big Creek	5
Manitowoc River	4.7
Little River	4.2
Pigeon River	3.2
Menomonee River	3
Menominee River	2.9*
Pike River	2.7
Whitefish Bay Creek	1.6
Root River	0.5
Mud Lake Drainage	0.1
Little Manitowoc River	0.1
Silver Creek	0.1
Stony Creek	0.1
Peshtigo River	0.1

*Steams at which round gobies were found immediately downstream of a physical barrier (dam or waterfall).

Azov seas, as well as several of their larger tributaries (Charlebois et al. 1997), and have since invaded other larger rivers in Eurasia (Brown and Stepien 2008). Smaller tributaries in Eurasia may also be occupied by round gobies, but round goby ascension into flowing water has been reported to be limited and much slower than other Ponto-Caspian gobiids such as the tube-nosed goby (*Proterorhinus marmoratus*) and monkey goby (*Neogobius fluviatilis*) (Harka and Bíró 2007). In the Great Lakes, round gobies have been reported in a handful of small tributaries to Lake Erie (Phillips et al. 2003; Krakowiak and Pennuto 2008), several tributaries in Ontario known as species-at-risk hot spots (Poos et al. 2009), and have been introduced by anglers to a few other inland streams (Carman et al. 2006). Our data illustrate the pervasiveness of this species in a variety of stream habitats and suggest that round gobies are more widespread and abundant in streams than previously thought.

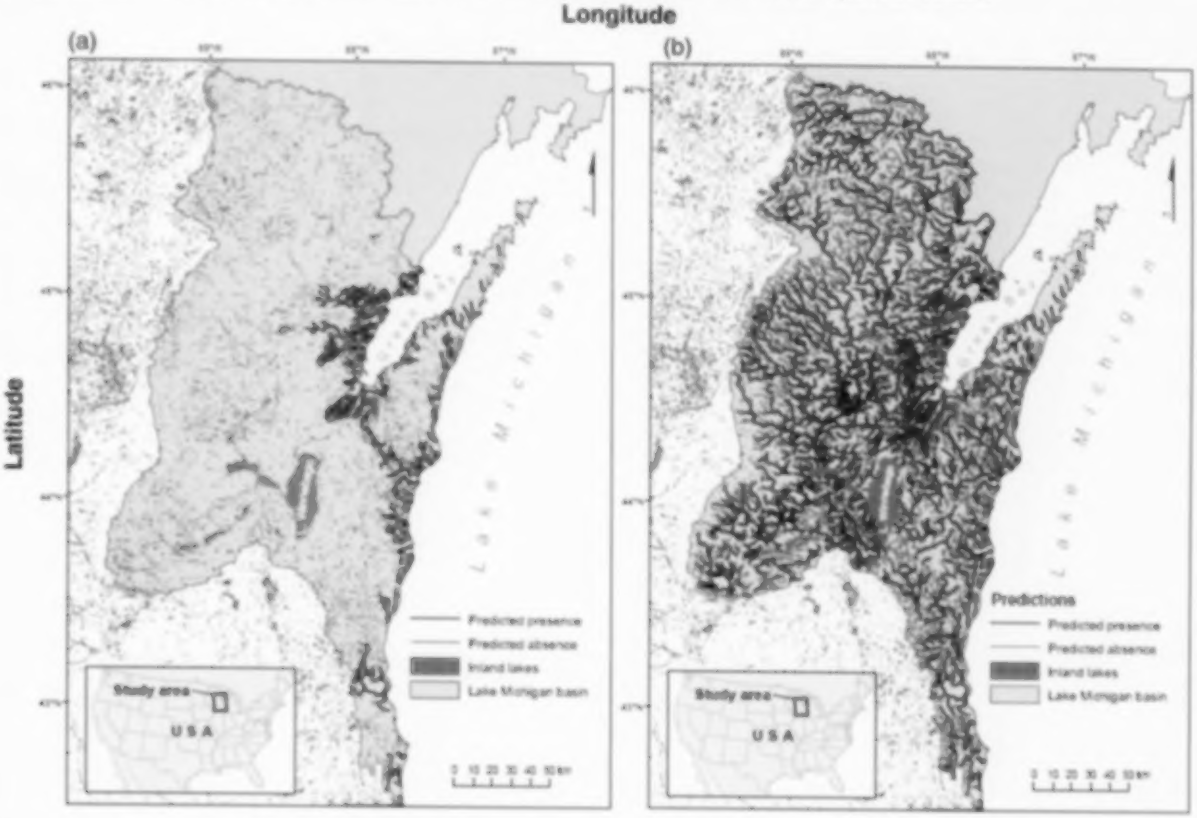
Our findings contribute to a growing body of evidence that species may not be limited to their native range habitats in invaded regions. In addition to invading stream habitat, round gobies can thrive in systems devoid of *Dreissenid* mussels (Carman et al. 2006), which are important forage of round goby in their native range and in the Great Lakes (Ghedotti et al. 1995; Ray and Corkum 1997; French and Jude 2001). Our results corroborate Carman et al. (2006), as *Driessenid* mussels were found at only 13% of sites where we detected round gobies. Other examples of species behaving unexpectedly in their invaded range include the rusty crayfish (*Orconectes rusticus*), which is native to streams in the Ohio River drainage, yet has prospered in north temperate lakes (Olden et al. 2006; Roth et al. 2007).

Ecological niche modeling, a tool commonly used to forecast species invasions, often assumes that species will establish in habitats similar to that of their native range (Peterson 2003). These studies also assume that a species is environment-limited in its native range and often only use presence data (instead of presence–absence data). Although this method has been used to predict the distributions of many invasive species (Peterson 2003; Thuiller et al. 2005; Mercado-Silva et al. 2006), the widespread round goby invasion of tributary streams would not have been predicted from native range data alone. Similarly, models based on invaded range data outperformed models based on native range data in forecasting the distribution of the New Zealand mudsnail (*Potamopyrgus antipodarum*) in Australia and North America (Loo et al. 2007) and cane toad (*Chaunus* [*Bufo*] *marinus*) in Australia (Urban et al. 2007). We encourage ecological niche modelers to include invaded range data when predicting species spread because species are not always limited to the habits and habitats of native range populations.

We found landscape-scale variables to be sufficient for describing and forecasting species distributions and detected no statistical difference between models that included landscape-scale and field-based data and models that included only landscape-scale data. In fact, our top three models (two including landscape-scale and field-based data and one including only landscape-scale data) were almost identical in their ability to predict round goby occurrence on independent data. This is useful because landscape-scale data are often easier to obtain than field-based data because of advances in spatial modeling techniques and availability of geographical databases (Wiley et al. 1997). Although fish distributions are often linked to field-based parameters such as habitat and temperature, landscape-scale variables such as watershed area, reach gradient, and geology are linked to fish habitat use and are dependable predictors of species presence–absence (Kruse et al. 1997; Porter et al. 2000; Creque et al. 2005). One study suggested that landscape-scale variables acting together could dictate field-based characteristics such as substrate and flow (Watson and Hillman 1997). Our results support these ideas, demonstrating that excluding field-based data does not necessarily result in inferior models.

This work is intended to help guide efforts to prevent the spread of harmful invasive species. Prevention is considered the most cost-effective strategy for dealing with species introductions (Leung et al. 2002; Keller et al. 2008). Upstream dispersal of round goby is likely occurring elsewhere in the Great Lakes basin, and the potential for human-assisted transport across stream barriers suggests that there are thousands of vulnerable streams in the region. The presence of round goby larvae in the water column provides a mechanism by which round gobies may be transported inland through water in the live wells of recreational boaters (Hensler and Jude 2007). The sizeable difference between the number of streams expected to be invaded by round gobies downstream of the first dams on Lake Michigan tributa-

Fig. 2. Forecasted distribution of round goby (*Neogobius melanostomus*) in tributary segments (*a*) downstream of the first impassable barrier and (*b*) ignoring the presence of impassable barriers. Black segments are predicted to be invaded by round goby and shaded segments are predicted to remain uninvaded by round goby. Predictions were made using a landscape-scale generalized linear mixed model for round goby presence–absence. Model was constructed based on data from 119 sites (excludes 11 sites upstream of dams).



ries and the number of streams suitable for round gobies regardless of dams highlights the importance of minimizing purposeful or accidental (bait-bucket or livewell) transport of round gobies. Round gobies have already been introduced to the Flint and Shiawassee rivers in inland Michigan (Jude 2001; Carman et al. 2006) and Lakes Simcoe and Rice in Ontario, Canada (Jason Borwick, Ontario Ministry of Natural Resources, Aquatic Research and Development Section, Laboratory of Fisheries Research, Third Floor North, 300 Water Street, Peterborough, ON K9J 8M5, Canada, personal communication, 26 September 2008), via bait-bucket transportation. Education efforts should be a key piece of the strategy to minimize further spread and impacts of round gobies and invasive species in general.

Efforts to increase stream connectivity by removing dams can also open new habitats to the natural dispersal of aquatic invasive species. Increasingly, decision makers are choosing to remove rather than repair aging and obsolete dams (Stanley and Doyle 2003). Although the ecological benefits of dam removals are well documented (Hart et al. 2002; Catalano et al. 2007; Doyle et al. 2008), invasive species add a level of complexity to improving stream connectivity. The removal of dams that are the first barrier between a Great Lake and its upstream watershed, for example, would open up new stream habitats not only to desirable fish species (Bednarek 2001; Bowman 2002), but also to aquatic invasive species. The general issue of stream connectivity facilitating invasive species spread is likely to be relevant in other regions of the world, though perhaps with a different set of invasive species.

Though efforts to forecast species distributions have done much to advance our understanding of species invasions, pre-invasion data on invaded ecosystems are necessary to answer questions involving ecosystem changes wrought by an invader (Hengeveld 1999). Pre- and post-invasion studies of ecosystems are rare because of the difficulty associated with predicting suitable systems (Hengeveld 1999), but results of this study provide such predictions for the round goby. Further work should address what effects round gobies have on native communities and energy pathways in streams, as such work has been done in the Great Lakes (Johnson et al. 2005) but not in tributaries (but see Krakowiak and Pennuto 2008). Further examination of potential biological barriers to round goby expansion (e.g., high predator abundance) would also be of great interest and would highlight ecological integration as a potential barrier to species invasions even when propagule pressure and habitat suitability are adequate for establishment.

This study provides strong evidence of the Great Lakes as a source population for aquatic invasive species to inland watersheds. We show that round gobies are thriving in relatively small tributary streams across a wide geographic scale, a surprising discovery considering that round gobies are usually associated with large lakes. Researchers who deal with predicting invasive species dispersal should not rely on native range data alone if invaded range data are available. Although there is much to be learned from the historical distribution of a species in its native range, only invaded range data incorporate unknown factors such as rapid adaptation and release from predators in forecasting the spread of an invader.

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Natal origin of Atlantic bluefin tuna (*Thunnus thynnus*) from Canadian waters based on otolith $\delta^{13}C$ and $\delta^{18}O$

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Abstract: Increased knowledge of stock mixing and migration of Atlantic bluefin tuna (*Thunnus thynnus*) is required to properly manage and conserve declining populations. Here, we predicted the nursery origin of giant bluefin tuna (n = 224) present in samples from Canadian waters using stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths. The isotopic composition of milled otolith cores (corresponding to the first year of life) of giant bluefin tuna from three decades (1970s, 1980s, 2000s) and three regions within or adjacent to the Gulf of St. Lawrence was compared with otolith $\delta^{13}C$ and $\delta^{18}O$ of yearling bluefin tuna collected from eastern (Mediterranean Sea – eastern Atlantic, n = 136) and western (western Atlantic, n = 103) nurseries. Maximum likelihood estimates indicated that greater than 99% of bluefin tuna in our Canadian samples originated from the western nursery. No significant differences in estimates of origin for bluefin tuna were detected among decades or among regions, suggesting little to no mixing of eastern and western populations in the Canadian samples examined. These findings justify the use of catch rates from the Gulf of St. Lawrence area as an index of abundance for the oldest members of the western population.

Résumé : Il est nécessaire de mieux connaître le mélange des stocks et la migration des thons rouges (*Thunnus thynnus*) de l'Atlantique afin de mieux gérer et préserver ces populations en déclin. Nous avons déterminé la nourricerie d'origine de thons rouges géants (n = 224) provenant d'échantillons tirés des eaux canadiennes à l'aide des isotopes stables $\delta^{13}C$ et $\delta^{18}O$ dans les otolithes. La composition de cylindres centraux moulus d'otolithes (correspondant à la première année de vie) de thons rouges géants de trois décennies (les années 1970, 1980 et 2000) et de trois régions intérieures ou adjacentes du golfe Saint-Laurent a été comparée aux $\delta^{13}C$ et $\delta^{18}O$ des jeunes thons rouges de l'année récoltées dans les nourriceries orientales (Méditerranée – est de l'Atlantique, n = 136) et occidentales (ouest de l'Atlantique, n = 103). Les estimations de vraisemblance maximale indiquent que plus de 99 % des thons rouges dans les échantillons canadiens proviennent des nourriceries occidentales. Il n'y a pas de différence décelable dans les estimations d'origine des thons rouges entre les décennies ou entre les régions, ce qui laisse croire qu'il y a peu ou pas de mélange entre les populations orientales et occidentales dans les échantillons canadiens examinés. Ces constatations justifient l'utilisation des taux de capture dans la région du golfe du Saint-Laurent comme indices d'abondance pour les membres les plus âgés de la population occidentale.

[Traduit par la Rédaction]

Introduction

Efforts to regulate Atlantic bluefin tuna (*Thunnus thynnus*) populations are headed by the International Commission for the Conservation of Atlantic Tunas (ICCAT). Bluefin tuna are currently managed as two separate stocks in the Atlantic Ocean, divided at 45°W longitude. The two-stock management strategy is based on eastern and western populations spawning in the Mediterranean Sea and Gulf of Mexico, respectively, with the eastern population being 10-fold larger than the western population (ICCAT 2006). Recent estimates from ICCAT suggest that the western spawning stock biomass (SSB) has fluctuated between 18% and 27% of the population level in the 1970s, and the eastern SSB is at less than 40% (ICCAT 2008). In response, ICCAT recommends substantially reducing fishing mortality in the near future to reduce the risk of fishery collapse and rebuild stocks (ICCAT 2008). Despite being managed by ICCAT for several decades, bluefin tuna populations have not recovered, leading many to question current stock assessments and the biological data used in population models.

Uncertainty regarding the population structure and degree

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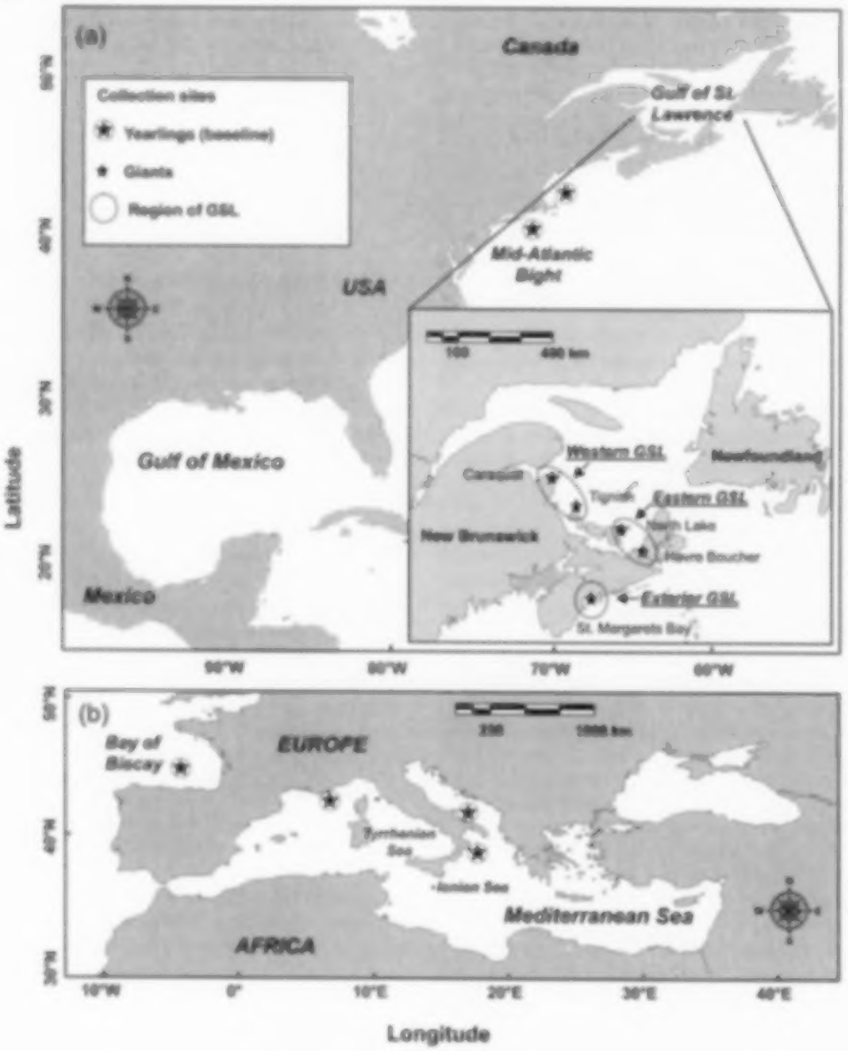
Fig. 1. Collection areas of yearling (baseline sample) and giant Atlantic bluefin tuna (*Thunnus thynnus*) in the (*a*) western and (*b*) eastern Atlantic Ocean and associated seas. Inset in western Atlantic plot shows the five ports from which otoliths of giant bluefin tuna were obtained from Canadian waters. Regional groupings (eastern, western, and exterior) of ports within and adjacent to the Gulf of St. Lawrence (GSL) are also denoted.


(a)
Canada
Collection sites
Yearlings (baseline)
Giants
Region of GSL
Gulf of St. Lawrence
Mid-Atlantic Bight
USA
100
400 km
Gulf of Mexico
Western GSL
Newfoundland
Caraquet
Tignish
Eastern GSL
North Lake
New Brunswick
Havre Boucher
Mexico
Exterior GSL
St. Margarets Bay
50°N
40°N
30°N
20°N
Latitude
90°W
80°W
70°W
60°W
(b)
Bay of Biscay
EUROPE
250
1000 km
Tyrrhenian Sea
Ionian Sea
Mediterranean Sea
AFRICA
50°N
40°N
30°N
10°W
0°
10°E
20°E
30°E
40°E
Longitude

of mixing between eastern and western populations is a major concern of assessment scientists. Evidence indicates that mixing occurs off the North American continental shelf from the Mid-Atlantic Bight to the Scotian Shelf and possibly extending to the Flemish Cap (Block et al. 2005; Rooker et al. 2007). However, the degree of mixing over time in this broadly defined foraging zone, which includes important fishing grounds of bluefin tuna from the Gulf of St. Lawrence to the Mid-Atlantic Bight, is unresolved. It is possible that contribution rates of eastern and western migrants are region- and time-specific within the western Atlantic, and thus population composition may vary spatially and temporally. Contemporary assessments of the Gulf of St. Lawrence fishery suggest that the region is comprised primarily of bluefin tuna from the western population (Rooker et al. 2008*b*); however, estimates of fidelity or stock structure over longer time periods are needed to assess natural variation in contribution rates to this region.

The Gulf of St. Lawrence has supported a rod-and-reel fishery for giant bluefin tuna for over 40 years (Mather et al. 1995), and catches from this region represent approximately 15% of the total allowable catch for the western stock (ICCAT 2006). The long-term consistency of catches dominated by giant bluefin tuna in the Gulf of St. Lawrence fishery has led to its use by ICCAT as an index of western stock abundance (ICCAT 2006). However, data from the Gulf of St. Lawrence fishery may not accurately capture population trends of the western stock if contribution rates of eastern and western bluefin tuna vary over time. Therefore, information on temporal or spatial changes in the stock

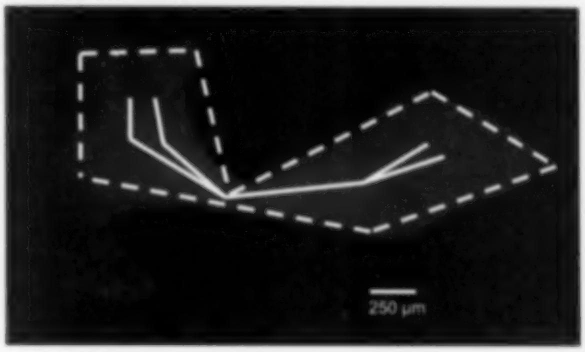
Table 1. Number of Atlantic bluefin tuna (*Thunnus thynnus*) otoliths collected at each sampling location each year from Canadian waters. All samples were from the Gulf of St. Lawrence, with the exception of those from St. Margaret's Bay (Nova Scotia).

	Year												
Location	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	2006	2007	Total
Caraquet	8	10	10	8	8								44
Havre Boucher							4						4
North Lake	12	10	12	9	10	1					11	20	85
St. Margarets Bay	5	7	14	11	4	3	5						49
Tignish								7	31	4			42
Total	25	27	36	28	22	4	9	7	31	4	11	20	224

structure of bluefin tuna in the Gulf of St. Lawrence is critically needed, particularly if this region represents an essential foraging ground of the vulnerable western population.

This study used stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios in otoliths as markers of natal origin for bluefin tuna from Canadian waters within or adjacent to the Gulf of St. Lawrence. Stable isotope signatures in milled otolith cores of giant bluefin tuna were compared with yearling (age-1) signatures from eastern and western nurseries (established baseline in part from Rooker et al. 2008*a*) to determine whether individuals originated from spawning grounds in the Mediterranean Sea or the Gulf of Mexico. Interdecadal and spatial variations in contribution rates to the Gulf of St. Lawrence fishery were also assessed to investigate temporal changes in mixing by predicting the origin of bluefin tuna collected in the 1970s, 1980s, and 2000s among multiple regions. Our working hypothesis is that the western population shows fidelity to the Gulf of St. Lawrence foraging ground, and thus contribution rates will be markedly higher for the western population over all three decades.

Fig. 2. Transverse section of otolith from a yearling Atlantic bluefin tuna (*Thunnus thynnus*) with preprogrammed drill path used to mill core material (age-1 period): continuous lines represent the micromill drill path to be powdered; broken lines highlight the otolith material collected within the approximate 250 µm distance that the drill bit will reach.



Materials and methods

Otolith collecting and processing

Otoliths from yearling bluefin tuna (approximately 50–80 cm fork length) were collected from eastern (eastern Atlantic and Mediterranean Sea, $n = 23$) and western (western Atlantic, $n = 22$) nurseries from 2006 to 2007 and combined with samples from 1999 to 2004 (eastern, $n = 113$; western, $n = 81$; Rooker et al. 2008*a*). Archived otoliths of giant bluefin tuna (mature adults >141 kg) collected in the Gulf of St. Lawrence (GSL) and St. Margaret's Bay (Scotian Shelf) from 1975 to 1984 were provided by Fisheries and Oceans Canada ($n = 193$; Fisheries and Oceans Canada, St. Andrews Biological Station, St. Andrews, New Brunswick). In addition, giant bluefin tuna were collected from the Gulf of St. Lawrence fishery out of North Lake, Prince Edward Island, in 2006 and 2007 (Rooker et al. 2008*b*; $n = 31$). Samples were representative of five fishing ports, which were grouped into three regions (western GSL (Caraquet and Tignish), eastern GSL (Havre Boucher and North Lake), exterior GSL (St. Margarets Bay); Fig. 1; Table 1). Sagittal otoliths were removed from all bluefin tuna, and fork length (cm) and weight (kg) were recorded for each specimen. Approximately 98% of measured tuna were 250–300 cm fork length (>15 years of age) for archived samples, and 90% were 230–270 cm fork length (>12 years of age) for modern samples (ages based on Rooker et al. 2007).

A single sagittal otolith (right or left) was randomly selected from each pair for analysis. Selected otoliths were cleaned of excess tissue and rinsed with deionized water (dH_2O). For yearling bluefin tuna (1999 to 2004), the entire otolith was powdered using a mortar and pestle to obtain material deposited during the first year of life. Otoliths from adults (and 2006 to 2007 yearlings) were embedded in Struers epoxy resin (Struers A/S, Ballerup, Denmark) for further processing and milling. Comparisons of whole versus milled otoliths were examined with otolith pairs from the same individuals, and no effect was detected (paired *t* tests, $p > 0.05$), indicating that core signatures did not differ between direct powdering and embedding–milling techniques (Rooker et al. 2008*b*). A 1.5 mm thick section of resin containing the otolith core was cut along a transverse plane using a Buehler IsoMet saw (Beuhler, Lake Bluff, Illinois) and was then attached to a sample plate using Crystalbond thermoplastic glue (SPI Supplies/Structure Probe Inc., West Chester, Pennsylvania). The region corresponding to the first year of growth (identified from measurements of transverse sections of otoliths from yearling bluefin tuna; Fig. 2) was isolated and powdered using a New Wave Research MicroMill (Fremont, California). A series of drill passes was run over a preprogrammed drill path by a 500 µm diameter Brasseler carbide bit (Brasseler USA, Medical L.L.C., Ventura, California) until a depth of approximately 750 µm was reached. Powder was collected with a microspatula and loaded into sample trays. All sampling equipment was

Table 2. Summary data for $\delta^{13}C$ and $\delta^{18}O$ concentrations from otoliths of giant Atlantic bluefin tuna (*Thunnus thynnus*) collected from Canadian waters and yearling bluefin tuna from the eastern and western Atlantic Ocean.

	1970s		1980s		2000s		All adults		Eastern yearlings	
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
Number	138	138	55	55	31	31	224	224	136	136
Minimum	−10.01	−2.2	−9.30	−1.9	−9.84	−1.9	−10.01	−2.2	−9.82	−1.5
Maximum	−7.26	−0.9	−7.27	−1.1	−7.32	−1.0	−7.26	−0.9	−7.20	−0.4
Mean	−8.59	−1.6	−8.32	−1.5	−8.37	−1.5	−8.50	−1.5	−8.44	−0.9
SD	0.47	0.2	0.44	0.2	0.60	0.2	0.50	0.2	0.42	0.2
	Eastern GSL		Exterior GSL		Western GSL		All adults		Western yearlings	
	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$
Number	89	89	49	49	86	86	224	224	103	103
Minimum	−10.01	−2.2	−9.35	−1.9	−9.84	−2.2	−10.01	−2.2	−9.78	−2.4
Maximum	−7.26	−1.0	−7.53	−0.9	−7.27	−1.1	−7.26	−0.9	−7.62	−0.9
Mean	−8.53	−1.6	−8.41	−1.5	−8.51	−1.5	−8.50	−1.5	−8.46	−1.6
SD	0.55	0.2	0.39	0.2	0.49	0.2	0.50	0.2	0.40	0.4

Note: Values for giant bluefin tuna are displayed according to the decades (1970s, 1980s, and 2000s) and regions (eastern, exterior, and western) from which samples were obtained. SD, standard deviation.

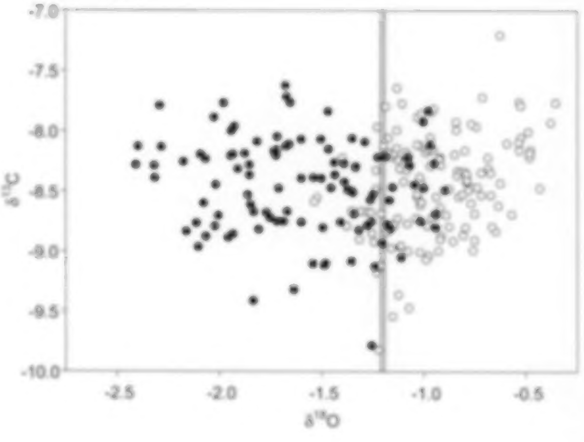
cleaned with 70% ethanol between samples to prevent cross contamination.

Otolith powders were analyzed for $\delta^{13}C$ and $\delta^{18}O$ using an automated carbonate preparation device (KIEL-III; Thermo Fisher Scientific, Inc., Waltham, Mass.) coupled to a gas chromatograph – isotope ratio mass spectrometer (Finnigan MAT 252; Thermo Fisher Scientific, Inc.) at the University of Arizona. Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70 °C. Analytical precision of the mass spectrometer was ±0.1‰ for $\delta^{18}O$ and ±0.06‰ for $\delta^{13}C$ (1 standard deviation, SD). Isotope ratios were calibrated based on repeated measurements of NBS-19 and NBS-18 and reported relative to the Pee Dee Belemnite (PDB) standard.

Data analysis

Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used to determine whether $\delta^{13}C$ and $\delta^{18}O$ in the otoliths of yearling and giant bluefin tuna differed among sampling regions and decades. Otolith $\delta^{13}C$ and $\delta^{18}O$ from yearling bluefin tuna were classified by quadratic discriminant function analysis (QDFA) and used as a baseline set for predicting the origin of giants from Canadian waters. Isotope signatures in cores of giant bluefin tuna differed significantly among decades (MANOVA, $p < 0.05$), suggesting the need to adjust for changes in $\delta^{13}C$ and $\delta^{18}O$ over time. Isotope signatures were adjusted for changes in ambient isotope concentrations by −0.0256‰ per year for $\delta^{13}C$ and 0.0043‰ per year for $\delta^{18}O$ (Schloesser et al. 2009). Both corrected and uncorrected otolith $\delta^{13}C$ and $\delta^{18}O$ from giants of unknown origin were compared with yearling baseline samples and classified to eastern or western nurseries using maximum likelihood estimates (MLE) from the mixed-stock analysis program HISEA as described by Millar (1990). HISEA was run under bootstrap mode to obtain standard deviations around estimated proportions with 1000 simulations. Hereafter, all reporting and testing of otolith $\delta^{13}C$ and $\delta^{18}O$ for giant bluefin tuna will be given as adjusted values unless noted otherwise.

Fig. 3. Otolith core $\delta^{13}C$ and $\delta^{18}O$ of yearling Atlantic bluefin tuna (*Thunnus thynnus*) collected from the eastern (open circle, n = 136) and western Atlantic (solid circle, n = 103) (in part from Rooker et al. 2008*a*). Shaded region shows gap between 1 standard deviation (SD) from the mean otolith $\delta^{18}O$ of yearlings from each nursery (−1.19 to −1.21).



Results

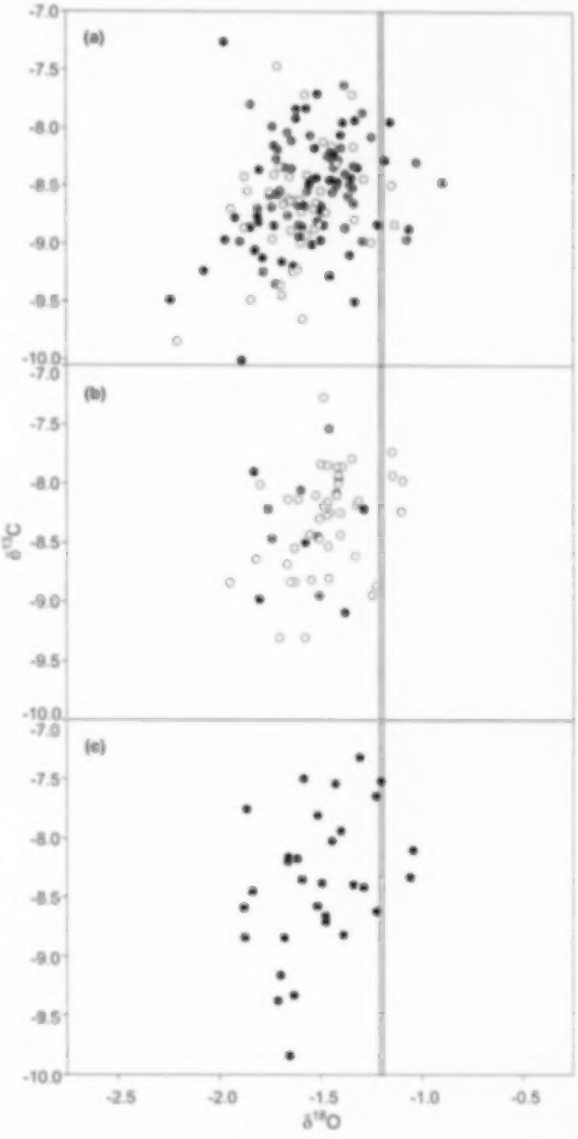
Isotope concentrations ($\delta^{13}C$ or $\delta^{18}O$) from otoliths of yearling bluefin tuna were distinct between eastern (eastern Atlantic and Mediterranean Sea) and western (western Atlantic) nurseries (MANOVA, $p < 0.05$; Fig. 3). Separation between nursery signatures was due to a significant $\delta^{18}O$ enrichment of 0.66‰ (ANOVA, $p < 0.05$) in eastern yearling otoliths (mean ± SD = −0.94‰ ± 0.25‰, n = 136) over western yearling otoliths (mean ± SD = −1.60‰ ± 0.39‰, n = 103) (Table 2). No significant differences were detected in otolith $\delta^{13}C$ between nurseries, with only a 0.02‰ enrichment in otolith $\delta^{13}C$ from western yearlings (mean ± SD = −8.46‰ ± 0.40‰, n = 103) over their eastern counterparts (mean ± SD = −8.44‰ ± 0.42‰, n = 136). Despite similar otolith $\delta^{13}C$ between nurseries, differences in $\delta^{18}O$ were great enough that cross-validated classification success from DFA was 84% among all year classes, with a 92% and 75% classification success for eastern and western yearlings, respectively.

No significant differences in adult otolith core $\delta^{13}C$ or $\delta^{18}O$ were detected among decades (MANOVA, $p > 0.05$) or regions (MANOVA, $p > 0.05$), which was expected given that interdecadal variation was accounted for in our adjustment. Mean otolith core $\delta^{13}C$ ranged from −8.59‰ to −8.32‰ across the three decades and did not differ significantly (ANOVA, $p > 0.05$) among the regions examined within or outside the Gulf of St. Lawrence (GSL): eastern GSL (mean = −8.53‰), exterior GSL (mean = −8.41‰), and western GSL (mean = −8.51‰) (Table 2; Fig. 4). Likewise, otolith core $\delta^{18}O$ was also similar among decades (ANOVA, $p > 0.05$), with means ranging from −1.6‰ to −1.5‰ in 1970 and 2000, respectively (Table 2; Fig. 4). No significant difference in otolith $\delta^{18}O$ was detected among regions (ANOVA, $p > 0.05$), with mean values ranging between −1.6‰ and −1.5‰.

MLE for all decades combined indicated that 100% of giant bluefin tuna in our Canadian samples originated from the western nursery, with no contribution from the eastern nursery (Table 3). The standard deviation around the western stock estimated proportion was <0.1% for all decades combined, meaning that there was a 68% probability that the actual contribution of the western nursery to the Gulf of St. Lawrence area was 100%. High classification (± SD) to the western nursery remained relatively consistent across decades: 1970s (100% ± 0.2%), 1980s (100% ± 0.1%), 2000s (99.6% ± 0.2%). Results from classifications of unadjusted values were nearly identical to those of adjusted values (Table 3).

Estimates of origin by region were also assessed using MLE, and the percentage of giants in our Canadian samples originating from the western nursery was greater than 99% in each of the three regions (Table 3). Western population contribution (± SD) was lowest in the exterior GSL region but still very high (99.3% ± 2.1%), indicating that only a small fraction (0.7%) of bluefin tuna in this region originated from the eastern nursery. Given that the standard deviation around the estimated proportions was 2.1%, there was a 68% probability that the eastern contribution of bluefin tuna in our sample ranged from 0.0% to 2.8%. Similarly, the contribution (± SD) of eastern Atlantic migrants to the other regions was also low: western GSL (0.0% ± 0.1%), eastern GSL (0.0% ± 0.2%). Necessary adjustments for decadal trends in isotope concentrations had little effect on classifications, with unadjusted signatures actually increasing the proportion of bluefin tuna classified to the western stock in the 2000 decade and exterior GSL region (Table 3).

Fig. 4. Otolith core $\delta^{13}C$ and $\delta^{18}O$ of giant Atlantic bluefin tuna (*Thunnus thynnus*) collected from Canadian waters in (*a*) 1970s ($n = 138$), (*b*) 1980s ($n = 55$), and (*c*) 2000s ($n = 31$). Values displayed according to sampling region: eastern (solid circle, $n = 86$), exterior (shaded circle, $n = 49$), and western (open circle, $n = 89$) Gulf of St. Lawrence. Shaded region shows gap between 1 standard deviation (SD) from the mean otolith $\delta^{18}O$ of yearlings from each nursery (−1.19 to −1.21).



Discussion

Otolith core $\delta^{13}C$ and $\delta^{18}O$ showed little variation among decades and regions. The lack of differences in stable isotope signatures among decades suggests that mixing rates of bluefin tuna migrating to the Gulf of St. Lawrence area have not changed during the time period covered. With over 99% of the bluefin tuna in the Gulf of St. Lawrence from the western population, it appears that a specific migratory contingent of the western population relies on this area as a feeding ground (Rooker et al. 2007). Furthermore, otolith $\delta^{13}C$ and $\delta^{18}O$ were similar among northern regions, suggesting that the bluefin tuna fishery examined in the Gulf of St. Lawrence is targeting a single contingent that migrates from the Gulf of Mexico spawning ground to forage in this

Table 3. Summary data for maximum likelihood classifications of giant Atlantic bluefin tuna (*Thunnus thynnus*) collected from Canadian waters.

	1970s		1980s		2000s		All	
	East	West	East	West	East	West	East	West
Adjusted classification	0.0	100	0.0	100	0.4	99.6	0.0	100
Adjusted SD	0.2	0.2	0.1	0.1	2.0	2.0	0.1	0.0
Unadjusted classification	0.0	100	0.0	100	0.1	99.9	0.0	100
Unadjusted SD	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
	Eastern GSL		Exterior GSL		Western GSL		All	
	East	West	East	West	East	West	East	West
Adjusted classification	0.0	100	0.7	99.3	0.0	100	0.0	100
Adjusted SD	0.2	0.2	2.1	2.1	0.1	0.0	0.1	0.0
Unadjusted classification	0.0	100	0.0	100	0.0	100	0.0	100
Unadjusted SD	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0

Note: Values are displayed according to the decades (1970s, 1980s, and 2000s) and regions (eastern, exterior, and western Gulf of St. Lawrence) from which samples were obtained. SD, standard deviation.

area. The lack of differences among regions also indicates that catches from the Gulf of St. Lawrence fishery are primarily composed of the western population, with little to no mixing with the eastern population. Therefore, the use of catches from the Gulf of St. Lawrence fishery as an indicator of western population abundance in assessments by ICCAT appears valid.

Isotopic signatures in the otolith cores of giant bluefin tuna from Canadian waters indicated that these individuals were almost exclusively of western origin, which differs from estimates of exchange in other parts of the western Atlantic. Evidence from tagging studies has shown mixing throughout the majority of the western Atlantic, including important foraging grounds from the Mid-Atlantic Bight to the Scotian Shelf (Block et al. 2005; Rooker et al. 2007). Additionally, otolith chemistry results from Rooker et al. (2008*b*) report that trans-Atlantic movement and population mixing is significant and size-dependent, with a substantial number of adolescent bluefin tuna collected in the US Atlantic originating from the Mediterranean Sea. These authors also reported that trans-Atlantic movement became more limited at the onset of sexual maturity. Thus, our finding of nearly 100% western bluefin tuna in the Gulf of St. Lawrence was not surprising given that all individuals were exclusively older, mature fish rather than adolescents. Our results suggest that mixing of giant bluefin tuna of eastern origin with their western counterparts at the northern extent of their range in the western Atlantic was exceptionally low for all three decades investigated.

Multiple studies have described migration routes of fishes and linked movement to specific environmental cues. It has commonly been hypothesized that physicochemical variables (i.e., temperature) influence migration pathways of bluefin tuna (Marsac 1999; Ravier and Fromentin 2004). In addition, Dodson (1988) hypothesized that bluefin tuna may intrinsically follow migration routes as a result of imprinting (natal homing) or spatial learning through schooling experiences (repeat homing). Repeated returns of bluefin tuna to spawning grounds supports natal homing (Rooker et al. 2008*b*) and spawning site fidelity (Teo et al. 2007), but driving forces for movements after spawning are poorly understood. Fromentin and Powers (2005) concluded that bluefin tuna are theoretically likely to perform repeat homing, with multiple studies showing that fish are capable of learning migration paths (Helfman and Schultz 1984; Mazeroll and Montgomery 1995) and experienced individuals can entrain behaviors in more numerous naïve fish (Laland and Williams 1997; Reebs 2000). Therefore, returns of giant bluefin tuna to the Gulf of St. Lawrence over the period investigated may be due to familiarity of the migration route by older, experienced individuals. If this hypothesis is true, declines in the numbers of adults making return trips to foraging grounds may alter migratory pathways of inexperienced bluefin tuna and ultimately influence where these individuals forage.

There are two documented accounts of extirpating bluefin tuna from regional fishing grounds without mass returns since, the Nordic fishery and the Brazilian episode (Fromentin and Powers 2005; MacKenzie and Myers 2007), both of which collapsed after large catches in the 1960s. The removal of bluefin tuna that were accustomed to Nordic or Brazilian migrations resulted in a loss of these migration routes. Removing key components of a population may also result in population bottlenecks that can jeopardize recovery efforts (Gardmark et al. 2003). Reduced population numbers are of particular concern for the smaller western population because there are only a few known feeding grounds for western bluefin tuna, and it appears likely that the Gulf of St. Lawrence serves as essential habitat for the western population. Consequently, the Gulf of St. Lawrence, as well as other critical habitats of bluefin tuna (i.e., Gulf of Mexico), must continue to be protected to ensure the sustainability of this fishery and the western population.

Acknowledgments

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The fire pulse: wildfire stimulates flux of aquatic prey to terrestrial habitats driving increases in riparian consumers

Rachel L. Malison and Colden V. Baxter

Abstract: We investigated the midterm effects of wildfire (in this case, five years after the fire) of varying severity on periphyton, benthic invertebrates, emerging adult aquatic insects, spiders, and bats by comparing unburned sites with those exposed to low severity (riparian vegetation burned but canopy intact) and high severity (canopy completely removed) wildfire. We observed no difference in periphyton chlorophyll *a* or ash-free dry mass among different burn categories but did observe significantly greater biomass of benthic invertebrates in both high severity burned and unburned reaches versus low severity burned reaches. Moreover, a significantly greater flux of adult aquatic insect emergence occurred at sites that experienced high severity fire versus low severity burned and unburned sites. The greatest number of spiders and bat echolocation calls were also observed at sites of high severity fire. Our results suggest that fires of different severity may have very different affects on stream-riparian food webs and that high severity wildfire may lead to an extended "fire pulse" that stimulates aquatic productivity and flux of prey to terrestrial habitats, driving local increases in riparian consumers.

Résumé : Nous examinons les effets à moyen terme de feux de friches (dans ce cas-ci, cinq années après le feu) d'intensités diverses sur le périphyton, les invertébrés benthiques, l'émergence des insectes aquatiques, les araignées et les chauves-souris en comparant des sites intacts à d'autres exposés à des feux de friches de faible intensité (végétation ripicole brûlée, mais canopée intacte) et de forte intensité (couvert végétal complètement disparu). Il n'existe pas de différence dans la chlorophylle *a* ou la masse sèche sans les cendres du périphyton entre les trois catégories d'exposition au feu, mais il y a une biomasse significativement plus importante d'invertébrés benthiques dans les sections sévèrement incendiées et les sections intactes que dans les sections légèrement brûlées. De plus, une émergence significativement plus importante d'insectes aquatiques adultes se produit aux sites qui ont connu un fort incendie par comparaison aux sites peu brûlés ou intacts. Le plus grand nombre d'araignées a aussi été observé dans les sites fortement brûlés et c'est là qu'on a enregistré le plus d'appels d'écholocation. Nos résultats laissent croire que les feux d'intensités diverses ont des effets très différents sur les réseaux alimentaires des rives des cours d'eau et que les feux de forte intensité peuvent produire une « impulsion » prolongée reliée au feu qui stimule la productivité aquatique et un flux de proies vers les habitats terrestres, ce qui explique les augmentations locales de consommateurs sur les rives.

[Traduit par la Rédaction]

Introduction

Wildfire affects a variety of ecosystems, from grasslands to forests, worldwide (Whelan 2007) and is among the most important natural disturbances. In the past several decades, the importance of fire as a significant agent in driving ecosystem structure and function has been increasingly recognized. Furthermore, the effects of wildfire may continue to grow as climate change drives increases in large wildfire frequency and longer fire seasons (Westerling et al. 2006). Many studies have investigated the role of wildfire in terrestrial systems (Agee 1993; Blair 1997; Bailey and Whitham 2002), and its effects on aquatic organisms and ecosystem processes has received some attention (Minshall et al. 1989; Gresswell 1999; Bisson et al. 2003). However, fewer studies have addressed the effects of wildfire on ecological linkages and the flow of resources from water to land.

Fire has the potential to alter water–land linkages, yet relatively few studies have addressed its influences on stream systems and the vectors of aquatic–terrestrial connectivity (Bisson et al. 2003; Dwire and Kauffman 2003). Streams and rivers are closely linked to their adjacent riparian zones and upland habitats by flows of material and organisms. Land-to-water fluxes including nutrients, leaves, and woody debris are well documented (Allan and Castillo 2007), and new work has highlighted the role of terrestrial invertebrates as prey for fish (Wipfli 1997; Nakano et al. 1999; Baxter et al. 2007). Recently, it has been demonstrated that flows of

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resources from water to land can fuel terrestrial food webs (Polis et al. 2004). In particular, the emergence of adult insects from streams constitutes a substantial export of energy to terrestrial consumers such as birds, bats, lizards, and spiders (Power and Rainey 2000; Nakano and Murakami 2001; Sabo and Power 2002). As a result of such connectivity, streams and their adjacent riparian zones are coupled in their responses to natural disturbances, including wildfire.

The effects of wildfire on stream-riparian ecosystems are thought to vary with time after a fire. Minshall et al. (2004) categorized the four stages of response and recovery following fire in watersheds in the western US as (*i*) immediate (the time of active burning to a few days after), (*ii*) short-term (a few days to the beginning of spring runoff), (*iii*) midterm (usually from spring runoff of the first postfire year to sometime beyond the 10th year), and (*iv*) long-term (occurring decades or centuries later). Most studies of effects of wildfire on stream-riparian ecosystems have focused on immediate and short-term time period responses. Understanding the role of wildfire in watersheds also requires investigation of mid- to long-term responses to this natural disturbance.

In the midterm time frame, wildfire has the potential to stimulate aquatic productivity. In contrast to immediate and short-term effects, in the midterm, wildfire may contribute to greater biomass and production of stream insect larvae (Minshall et al. 1989). The basis for this increase in production may be faster algal growth in streams as a result of canopy removal, higher temperatures, or increased delivery of inorganic nutrients such as nitrogen and phosphorus (Spencer and Hauer 1991; Robinson et al. 1994; Minshall et al. 1997). An increase in stream primary production may drive increases in secondary production of herbivorous stream insect larvae (Minshall 2003). On the other hand, effects of fire might diminish zoobenthos via habitat disturbance (e.g., debris flows, increased sedimentation). If the production of benthic insect larvae were stimulated, this should translate into more adult insects emerging from streams in burned vs. unburned sites.

Consumers specializing on riparian insects may also benefit from a pulse in aquatic productivity following wildfire. A large portion of emerging insects do not return to streams (Jackson and Fisher 1986) and can contribute from 25% to 100% of the energy or carbon required by many riparian consumers (Baxter et al. 2005). For example, some guilds of spiders derive the majority of their carbon from emerging stream insects (Power and Rainey 2000; Collier et al. 2002; Paetzold et al. 2005). In particular, spiders of the family Tetragnathidae build horizontal webs in riparian zones and are dependent on emerging insects for a large portion of their diet (Williams et al. 1995; Kato et al. 2004), and their abundance tracks spatial variation in insect emergence (Kato et al. 2003; Iwata 2007; Marczak and Richardson 2007). Similarly, bats that forage on emerging insects have also been shown to track changes in the availability of this resource (Power and Rainey 2000; Fukui et al. 2006). Therefore, if wildfire drives increases in prey availability, this may lead to local increases of these terrestrial insectivores.

If wildfire affects the flux of aquatic prey to terrestrial consumers, the severity of fire may mediate the strength of such a response. For example, in terrestrial systems, more severe fires have stronger effects on forest and grassland structure and composition (Agee 1993; Halpern and Spies 1995; Dwire and Kauffman 2003). Furthermore, the response to fire severity can be incremental, with high severity fires having the greatest effects and those of low severity fire falling intermediate (see Halpern and Spies 1995). In some cases, severe fires may be more likely than mild fires to increase both primary and secondary productivity and affect higher trophic levels (see Bailey and Whitham 2002). Furthermore, pulses in productivity are common following infrequent fires in tallgrass prairie systems (Seastedt et al. 1991; Blair 1997). Just as fire severity has consequences for terrestrial ecosystems, it may also affect responses at the aquatic–terrestrial interface. Thus, there is a need for studies that explicitly investigate the effects of fire severity on linkages between stream and riparian ecosystems.

In this study, we sought to examine the midterm (5–10 years after the fire) effects of wildfire on algae, the production of stream insects, and consequences for riparian predators of these insects. We hypothesized that wildfire results in an extended "fire pulse" by stimulating aquatic production and the flux of aquatic prey to terrestrial habitats, driving an increase in riparian consumers in the midterm period. To test this hypothesis, we compared various food web components in a suite of unburned watersheds with those that had experienced low severity and high severity wildfire. We measured responses across three trophic levels: biomass of periphyton (attached stream algae), benthic insects and emergence of adult aquatic insects, and occurrence of spiders and bats. We tested four predictions: (*i*) biomass of periphyton would be greater in burned vs. unburned sites, (*ii*) biomass of benthic insects would be greater in burned vs. unburned sites, (*iii*) the flux of emerging adult aquatic insects would be greater in burned vs. unburned sites, and (*iv*) the frequency of occurrence of spiders and bats would be greater at burned vs. unburned sites. In addition, we hypothesized that the effects of wildfire would be additive such that sites exposed to high severity fire would have the highest periphyton biomass, stream insect productivity, and occurrence of riparian consumers, whereas low severity burned sites would fall intermediate relative to unburned sites. Thus, in this field study, we examined the effects of fire and fire severity on both aquatic and terrestrial organisms. Most studies of wildfire effects have tended to measure either one factor among sites or many factors at one site, whereas this investigation addressed effects on multiple trophic levels across 16 sites.

Materials and methods

Study site

Study streams were located within the Payette National Forest in central Idaho, USA, on tributaries of Big Creek, which flows into the Middle Fork of the Salmon River in the Frank Church "River of No Return" Wilderness Area. We chose to study the effects of wildfire in this wilderness watershed because we wanted to measure responses by stream ecosystems mostly unaltered by humans and also because here natural fire regimes have been minimally affected by fire suppression or land cover change. The streams flow through steep, narrow valleys with forested slopes, a mixture of primarily Douglas-Fir (*Pseudotsuga menziesii*) and Ponderosa Pine (*Pinus ponderosa*). Additionally, bare and sparsely vegetated areas of grass and sagebrush (*Artemisia*) are common. The dominant riparian vegetation includes Red Osier Dogwood (*Cornus sericea*), Rocky Mountain Maple (*Acer glabrum*), and Alder (*Alnus viridis*). The region receives an average of 40 cm of precipitation annually, the majority falling as snow, resulting in peak flows from late spring through midsummer. The streams generally remain at baseflow conditions from late summer through winter.

We selected study streams that were affected by the extensive Diamond Peak wildfire, which burned most of the lower portion of the Big Creek drainage (including upland and riparian zones) to varying degrees in 2000. Based on the relative area burned upstream and the condition of post-fire riparian vegetation in 2005, we categorized reaches in tributaries of the lower Big Creek drainage into three classes (Fig. 1): (*i*) high severity burned, where wildfire removed canopy from large areas of the catchment upstream and removed both riparian understory and canopy such that only the understory had returned by 2005; (*ii*) low severity burned, where fire burned less catchment area and mainly removed the understory such that the understory had returned under an intact canopy; and (*iii*) unburned, where the upstream catchment and reach were entirely unburned or burned long ago (>50 years). Because of the spatial extent and high severity of the fire, our study design was necessarily unbalanced, with six high severity burned reaches, three low severity burned reaches, and three unburned reaches. Six high severity burned sites were selected to increase statistical power, but it was impossible to find additional unburned and low severity burned study reaches close enough to the study area for repeated sampling. For the field season of 2006, four more reaches, two unburned (second and third order) and two high severity burned (third and fifth) (Supplemental Table S1, available from the NRC Data Depository[3]), were included in the study to make additional measurements of periphyton and spider abundance. Within the three types, we selected 100 m study reaches and used segment and reach classification (sensu Frissell et al. 1986; Bisson et al. 2006) to identify sites that were similar in stream size, valley segment type, and reach type. All were second- to third-order streams in alluviated canyon segments with step-pool reach types. In addition, sites were located in the lower portion of each tributary to facilitate access via hiking.

Sampling

We sampled 12 reaches from May through October 2005, with additional samples collected on four more reaches for a total of 16 reaches in June and July 2006. At each site, we collected data on a suite of physical and biological parameters as described below following the methods of long-term monitoring studies conducted in this area (see Davis et al. 2001).

We collected data on a range of physical factors that we anticipated might affect production within the linked aquatic–terrestrial food web. These habitat variables included solar radiation, aspect, temperature, stream width and depth, streambed substrate conditions, and available structure for spider web attachment. Influx of solar energy can be an important factor in determining the productivity of a stream. We estimated solar input at each site between 1200 and 1400 on cloudless days during July 2006. Potential solar input was measured at four locations along each reach, every 25 m, using a Solar Pathfinder, which measures the available annual solar radiation as constrained by topographic features (Solar Pathfinder, Linden, Tennessee; www.solarpathfinder.com). In addition, we took 20 measurements of photosynthetically active radiation (PAR; model LI-1000, LI-COR, Lincoln, Nebraska) every 2 m just above the water surface beginning at the downstream end of each 100 m reach. We took the measurement within the dominant type of lighting present (i.e., direct sunlight vs. shade) along the width of the stream at each transect, taking into account the presence or absence of vegetation. We monitored water temperature at each site year-round using StowAway Tidbit and HOBO data loggers (Onset, Pocasset, Massachusetts) as it is known to affect aquatic invertebrate species composition, life history (including timing of emergence), and production (Huryn and Wallace 2000). We also monitored water temperature during the summer of 2006 at the additional four sites. Because the condition of the stream bed can influence the production of algae and invertebrates (Allan and Castillo 2007), we measured depths and widths of the stream channels and completed substrate surveys for each reach. At 50 locations (every 2 m) along each 100 m study reach, a rock was randomly selected, its size was measured, and percent embeddedness was estimated (Davis et al. 2001). To survey potential habitat for attachment of spider webs, we measured stream distance between vegetation over the channel and counted availability of wood (Laeser et al. 2005).

We measured stream periphyton from five rocks haphazardly selected from within riffles distributed systematically (approximately every 25 m) throughout each study reach. We scraped an area of 2.96 cm^2 of each rock and analyzed biomass as chlorophyll *a* and ash-free dry mass. We extracted chlorophyll *a* following standard methods (in 10 mL of methanol, filtered through Watman GF/F 4.7 cm filters), and estimated biomass using a spectrophotometer (model ThermoSpectronic Genesys 5, Thermo Fisher Scientific Inc., Waltham, Mass.) (American Public Health Association (APHA) 1995). Following extraction, periphyton samples were ashed at 550 °C, rewetted, dried at 105 °C, and weighed for ash-free dry mass (APHA 1995). We substituted 2005 data for two unburned and two low severity burned reaches in the analysis of periphyton samples because a wildfire in July 2006 burned study sites before sampling could be completed. Periphyton data from 2005 and 2006 were compared for all sites from which data were col-

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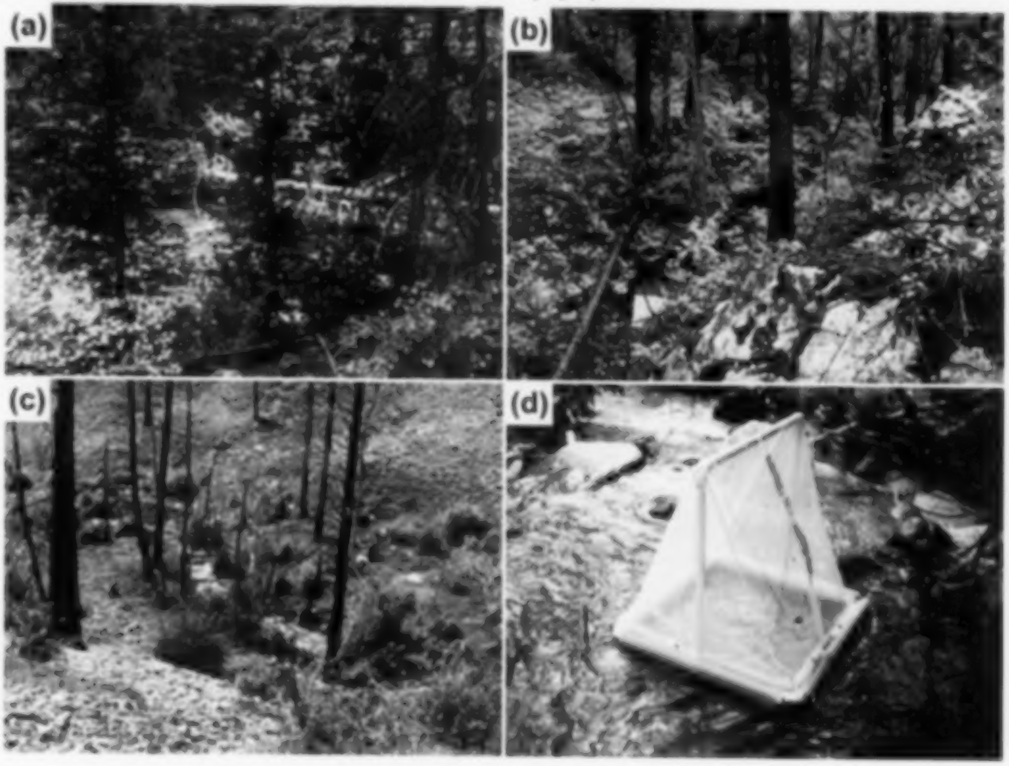
[3] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5357. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Fig. 1. (*a*–*c*) Pictures of the three fire classes determined based on existing postfire vegetation in 2005: (*a*) unburned, within the last 50 years; (*b*) low severity burned, where fire burned riparian vegetation (as evidenced by the fire scar on the base of the tree located at the center of the image) but the canopy remained intact; and (*c*) high severity burned, with both riparian vegetation burned and canopy removed by wildfire. (*d*) Example of floating emergence trap used to collect emerging aquatic insects.



lected in both years, and no difference in periphyton biomass between years was detected.

We used a Surber sampler (0.093 m^2; Aquatic Research Instruments, Hope, Idaho) to sample zoobenthos once in July 2005 by collecting five replicates from random locations within riffles every ~20 m in each study reach. We measured the flux of adult aquatic insects emerging from each reach from May through October 2005 using four 0.33 m^2 floating emergence traps (Fig. 1) set on the stream surface. Traps were placed in pools to create a solid seal with the water surface as riffles experience more turbulence and, additionally, because pools may be where most emergence occurs (Iwata 2007). Each set of traps accumulated insects continuously for 4–5 days prior to collection from May through August and were sampled on a biweekly basis from late August through October. We removed insects from the traps using an aspirator.

We sorted all benthic and adult insects and identified them to the lowest level required to classify them into trophic guilds and major life history types (usually family or genus). The latter was done mainly to address potential responses of community composition to wildfire (Malison 2008). We dried ($105\ ^\circ C \geq 24$ h) and weighed all insects (to the nearest 0.001 g) to obtain estimates of biomass for each taxon. Floating emergence traps underestimate the number of adults of bank-emerging taxa (Wallace and Anderson 1996; Stewart and Stark 2002), which in these streams principally include relatively low numbers of stoneflies (Plecoptera; Malison 2008). These were rarely captured in our traps, and we omitted these underestimated taxa from our analyses. As insects were washed out of emergence traps during heavy rain, we considered the sample collection time to be the total days during the sampling period without rain, excluding brief rain showers (which were insufficient to remove insects from netting).

With respect to predators of aquatic insects, we surveyed both spiders and bats. We surveyed web-weaving riparian spiders along 30 m transects encompassing both banks of each study reach during July when spiders were at peak abundance. We surveyed spiders by walking streambeds at night (2200–0000) when spiders were most active and visible. Spider abundance was recorded by family, which is easily distinguishable in the field. More than 90% of spiders belonged to the families Tetragnathidae and Araneidae, and the former were dominant. We conducted surveys of bats at night at three unburned and three high severity burned sites, once in June, July, and August 2005, in conjunction with the moon phase (not during full moon). We used passive acoustic monitoring to record the frequency of bat echolocation calls (AnaBat Bat Detector; Titley Electronics, New South Wales, Australia). We did not survey low severity burned sites for bats because of the proximity of several of these sites to caves. Furthermore, because bats are highly mobile, we could not be sure that the distance between some low severity and high severity burned habitats was great enough that bats would not forage in both habitats during a short time period. Surveys started just after dusk and were at least 2 h in duration. We estimated bat foraging by counting the number of search and feeding buzz calls. We defined a call as the start to the end of sequential pulses ending in a silent period. Because buzz calls (characterized by an increased frequency and emitted during the terminal phase of preying on an insect) were not recorded frequently, we used the combined number of buzz and search calls to estimate foraging activity, as there is a strong association between the number of search and buzz calls (Fukui et al. 2006). The silver-haired bat (*Lasionycteris noctivagans*) was the dominant bat species in June, whereas several species of *Myotis* were more common in July and August (Jason Beck, Idaho State University, Department of Biological Sciences, 921 S. 8th Avenue, Pocatello, ID 83209, USA, unpublished data).

Statistical analysis

The effect of burn severity on all habitat characteristics was analyzed using one-way analysis of variance (ANOVA) (PROC GLM, SAS 9.1; SAS Institute Inc., Cary, North Carolina). We tested the effect of burn severity on periphyton (chlorophyll *a* and ash-free dry mass), biomass of benthic insects, and spider abundance using ANOVA (PROC GLM, SAS 9.1). In the analyses of benthic insects and spider occurrence, pairwise comparisons among the least squares means for burn type were assessed using Tukey's honestly significant difference (HSD). The effect of burn severity on flux of insect emergence ($mg \cdot m^{-2} \cdot day^{-1}$) was analyzed using repeated-measures ANOVA (PROC MIXED, SAS 9.1), using burn severity as a fixed factor and stream as a random factor (stream nested within burn type).

Sample periods, the unit of measurement, were repeated 22 times, but not all emergence traps started running at the same time in all drainages. Hence, because of missed observations in some weeks, we pooled every three to four samples into six sampling periods. In the repeated-measures analysis of emergence, we made two a priori comparisons (because of low power): between unburned and high severity burn categories and between unburned and low severity burn categories. These comparisons were assessed using Dunnett's test. A pairwise comparison among the least squares means using Tukey's HSD was used to compare emergence between low and high severity burn categories. We analyzed the bat echolocation response data using a *t* test because we had only two reach types — high severity burned and unburned. All biomass and count data were log-transformed to meet the assumptions of parametric analysis. *P* values below 0.05 were considered significant, and *P* values between 0.05 and 0.1 were considered marginally significant, though of potential biological meaning, given the low sample size and weaker statistical power of this field study.

Results

In general, habitat characteristics did not vary significantly among stream reaches in different burn severity categories (see Supplemental Tables S1 and S2[3]). Potential solar insolation (from Solar Pathfinder), stream substrate embeddedness, distance between overhanging vegetation, and amount of wood did not differ among burn severity categories ($F_{[2,8]} \leq 1.98$, $P \geq 0.200$; Supplemental Table S1[3]). Despite no statistical differences in measured potential solar insolation (shading not incorporated), there was a marginally significant difference in photosynthetically active radiation (PAR) readings at stream surfaces by burn category ($F_{[2,8]} = 4.02$, $P = 0.062$). Twofold greater PAR reached the stream surfaces at high severity burned vs. unburned sites (Supplemental Table S2[3]; 954 ± 211 vs. 458 ± 106 μmol; Tukey's HSD, $P = 0.360$), and almost seven times more reached the surface of high severity burned streams than low severity burned streams (Supplemental Table S2[3]; 954 ± 211 vs. 143 ± 71 μmol; Tukey's HSD, $P = 0.059$). Although there were differences in light, there were no differences in water temperature, as annual degree days did not differ by burn category (Supplemental Table S2[3]; 1923 ± 142 (unburned), 2335 ± 410 (low), and 2187 ± 86 (high); $F_{[2,9]} = 0.83$, $P = 0.471$). Low severity burned reaches had somewhat smaller widths and depths ($F_{[2,9]} \geq 2.72$, $P \leq 0.119$) and somewhat smaller median substrate size (Supplemental Table S1[3]; 10.5 ± 3.6 vs. 20.3 ± 2.7 and 18.7 ± 2.0 cm; $F_{[2,9]} = 3.27$, $P = 0.086$) than both the unburned and high severity burned reaches, though differences were only marginally significant.

Contrary to our hypothesis, we observed no difference in periphyton chlorophyll *a* ($F_{[2,13]} = 1.20$, $P = 0.308$) or ash-free dry mass ($F_{[2,13]} = 1.17$, $P = 0.316$) among burn categories (Fig. 2*a*). Both the unburned and high severity burned sites were similar in chlorophyll *a* (Fig. 2*a*), but the low severity burn category had a slightly lower chlorophyll *a*. Both the unburned and high severity burned sites also had similar ash-free dry mass of periphyton (6.9 ± 1.2 vs. 7.5 ± 0.8 $g \cdot m^{-2}$), and the low severity burn category had a slightly lower ash-free dry mass (5.8 ± 1.2 $g \cdot m^{-2}$).

Although we did not observe a difference in periphyton biomass, there was a strong overall effect of burn type on benthic insect biomass ($F_{[2,9]} = 41.04$, $P < 0.0001$). In contrast to our incremental hypothesis, the greatest difference that we observed was actually between high and low severity burn categories, with high severity burned sites having almost fivefold the biomass of benthic insects of low severity burned sites (Tukey's HSD, $P < 0.0001$) versus 1.6 times the biomass of benthic insects of unburned reaches (Tukey's HSD, $P = 0.100$). The latter comparison was not significant because of the high variability in burned versus unburned reaches (Fig. 2*b*).

The ordinal magnitude of emerging insect flux in the three reach types was similar to that for zoobenthos, with high severity burned reaches having the greatest emergence, unburned reaches falling intermediate, and low severity burned reaches the lowest (Fig. 2*c*). There was a marginally significant overall effect of burn severity on aquatic insect emergence (ANOVA, $F_{[2,9]} = 4.03$, $P = 0.056$). This analysis was based on over 700 samples collected from 22 different sample periods and 12 streams during June through October 2005. The difference between unburned and low severity burned reaches was small (Dunnett, $P = 0.985$), whereas the flux of emerging insects was marginally significant and more than twofold greater in the high severity burn category than in the unburned category (Dunnett, $P = 0.093$). The

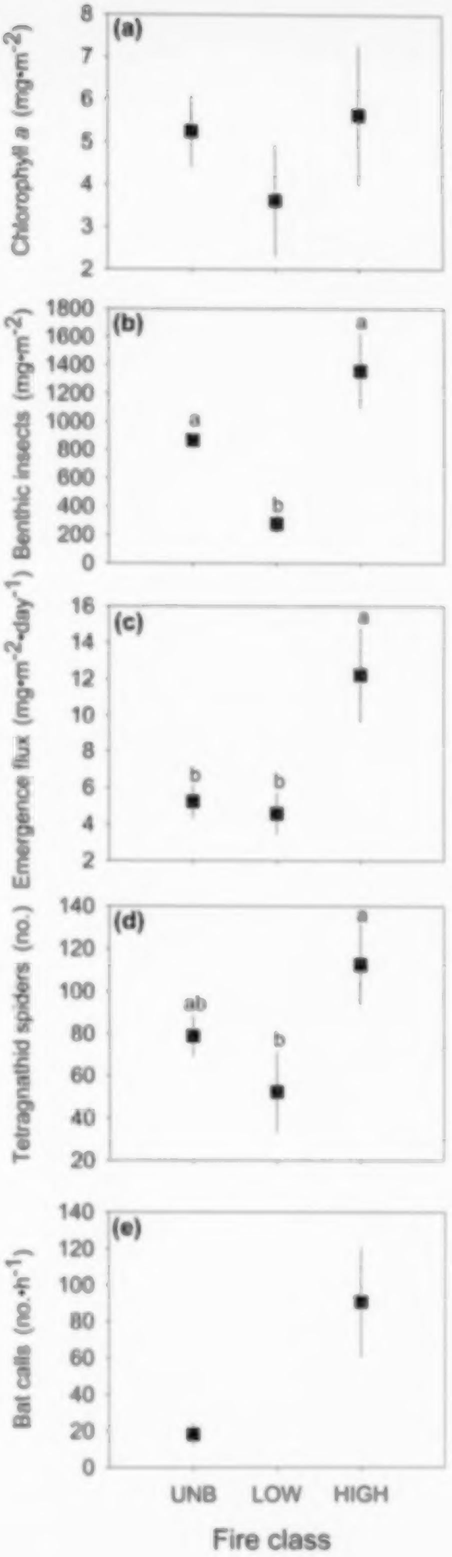


Fig. 2. Results of five food web components sampled from 16 streams between May and October 2005 in reaches of three fire classes: (*a*) periphyton chlorophyll *a*; (*b*) biomass of benthic insects; (*c*) emergence flux; (*d*) number of tetragnathid spiders; and (*e*) number of bat echolocation calls. Fire classes: UNB, unburned (n = 5 for chlorophyll *a* and spiders, n = 3 for benthic invertebrates, emergence, and bats); LOW, low severity burned (n = 3 for chlorophyll *a*, benthic invertebrates, emergence, and spiders); HIGH, high severity burned (n = 8 for chlorophyll *a* and spiders, n = 6 for benthic invertebrates and emergence, n = 3 for bats). Different letters indicate significant differences ($P < 0.1$) among burn types based on least significant difference (LSD) comparisons after analysis of variance (ANOVA) of log-transformed data. However, differences indicated in number of Tetragnathid spiders and between high severity burned and unburned emergence were marginally significant (P = 0.07 and 0.09, respectively).

flux of emerging insects was almost threefold greater in the high severity burn category than in the low severity burn category (Tukey's HSD, P = 0.036). We also observed a significant effect of time on emergence ($F_{[5,28]}$ = 6.74, P = 0.0003), with the greatest flux difference between burn categories occurring early in the summer during peak emergence. For example, emergence was up to fivefold greater in high severity burned sites than in both unburned and low severity burned sites during the month of June. Though the flux of emergence became more similar among burn categories by late July, this same pattern held throughout August, September, and October when total emergence was lower. There was no significant interaction between time and burn type ($F_{[10,28]}$ = 1.26, P = 0.301), indicating similar patterns of emergence in all streams.

We also observed effects of wildfire on insectivorous predators. The density of tetragnathid spiders differed by burn category, though the overall effect was only marginally significant ($F_{[2,13]}$ = 3.22, P = 0.073). The ordinal magnitude of the three burn categories was again the same as that for benthic invertebrates and emerging insects, but the difference was only marginally significant between high and low severity burned reaches (Fig. 2*d*; Tukey's HSD, P = 0.062). No significant differences were observed between unburned and high severity burn categories (Tukey's HSD, P = 0.55) or between unburned and low severity burn categories (Tukey's HSD, P = 0.32). In terms of vertebrate predators, the frequency of bat echolocation calls was greater in high severity burned reaches than in unburned reaches (Fig. 2*e*; *t* test, P = 0.04).

Discussion

The findings of this study suggest that high severity wildfire may drive a pulse in aquatic productivity detectable at multiple trophic levels, including the riparian consumers of aquatic insects. We observed the greatest biomass of benthic insects and emerging adult aquatic insects, occurrence of tetragnathid spiders, and frequency of bat echolocation calls in reaches that had been severely burned (forest canopy removed) 5 years prior to our study. Our results further indicate that fire severity may mediate such responses because the greatest differences that we observed were between high and low severity burned sites rather than between burned and unburned sites as we had predicted. Several studies have documented short-term responses to fires, including pulses of nutrients into streams directly following fire (Tiedemann et al. 1978; Schindler et al. 1980; Spencer and Hauer 1991). However, we observed that the effects of high severity wildfire could last longer, continuing at least 5 years after the fire with an extended pulse of stream insect productivity, as indicated by adult insect emergence. Rather than a flux from land to water, characteristic of short-term fire effects on nutrients, this midterm increase in productivity was dominated by an increase in aquatic insects emerging into riparian areas. Just as the input of wood from land into streams continues for many years after a fire (Minshall et al. 1989), providing important habitat for stream organisms (Gregory et al. 2003), this midterm pulse from water to land may serve to extend the temporal effects of wildfire, with potential consequences for terrestrial predators.

The greater emergence of adult stream insects from sites that experienced high severity wildfire appeared to be associated with higher local abundance of Tetragnathid spiders and bats. Factors other than aquatic insect prey such as fire-induced changes in habitat (e.g., more open foraging habitat for bats) or increases in terrestrial insect prey may have contributed to the patterns that we observed for spiders and bats. However, the latter is not likely, as a parallel study found terrestrial insects to be least abundant at high severity burned sites compared with those in the unburned and low severity burn categories (measured by pan traps; Breanne Jackson and Jeff Braatne, University of Idaho, College of Natural Resources, P.O. Box 441142, Moscow, ID 83844, USA, unpublished data). Experimental work has shown that these insectivores can track variation in emergence (Kato et al. 2003; Fukui et al. 2006), and we expect that the same may occur following severe wildfire. As observed by Nakano and Murakami (2001), such a subsidy can be especially important in periods when terrestrial food resources are less available for riparian insectivores. Though the magnitude of organic matter flux via emerging insects is small compared with that of inputs from land to water (Fisher and Likens 1973; Webster and Patten 1979), a large portion of this high quality prey does not return to streams (Jackson and Fisher 1986) and may be of disproportionate importance to terrestrial predators, including others that we did not investigate (e.g., birds, reptiles, and amphibians) (Baxter et al. 2005).

Just as the flood pulse (Junk et al. 1989) delivers terrestrial carbon to rivers while the land is subsidized by water and other materials, wildfire also results in amplified reciprocal fluxes between the land and water and its effects can extend many years after the event. Increased postfire productivity within streams also has the potential to fuel additional aquatic consumers such as predacious aquatic insects (Malison 2008) and fish (Dunham et al. 2007; Koetsier et al. 2007). In fact, we have observed (P. Della Croce and C. Baxter, unpublished data) greater export of drifting insects and aggregation of trout at confluences of high severity burned versus unburned tributaries, suggesting that fire-induced increases in insect productivity of tributaries might have important consequences for downstream fisheries.

The mechanisms responsible for the greater benthic invertebrate biomass and emerging insect flux in sites that experienced high severity wildfire are not clear. We initially hypothesized a bottom-up effect, i.e., an increase in consumers driven by an increase in primary producers. However, we observed no significant differences in periphyton biomass by burn severity category, despite the fact that there was a greater amount of light available at high severity burned sites. There may be a number of reasons why we did not observe these differences. First, the logistics of conducting this work in the wilderness precluded measurement of actual primary productivity, though this would have been a more sensitive measure than one snapshot of standing crop biomass. Though some studies have described increases in periphyton biomass in the short and intermediate time frames following fire (e.g., Minshall et al. 1997), less detectable effects in the midterm may require more sensitive measures of production and turnover rates, which have not yet been accomplished as part of wildfire studies. Though similar biomass was present in our study, it does seem likely that production of algae may have varied by burn severity category as a result of more rapid turnover of algae at high severity burned sites. Second, there may have been greater production of algae at high severity burned sites, but also greater insect grazing effects that may have resulted in similar biomass of periphyton in different burn categories. Third, there may have been differences among categories but our sampling method did not have sufficient power or precision to detect them. Lastly, there may not have been a difference in biomass or production of algae in the midterm period following wildfire in these streams because they were limited by something other than sunlight such as nutrients (Marcarelli and Wurtsbaugh 2007). Given the patchiness of periphyton growth in both space and time and that the pattern was similar to those that we observed for higher trophic levels (just not significant), it seems likely that we did not observe a pattern in periphyton biomass because of a combination of the first three reasons.

Another possible mechanism contributing to greater aquatic insect production at sites exposed to high severity wildfire may be that these sites receive increased inputs of allochthonous carbon in the midterm period. Allochthonous carbon is known to be a key resource for headwater stream invertebrates (Allan and Castillo 2007), and the regrowth of riparian vegetation following a fire is likely to include proportionately more herbaceous plants and deciduous shrubs and trees than might have been present before a fire (Dwire and Kauffman 2003). Consequently, there may be greater delivery of more labile (edible) plant material to streams at burned versus unburned sites, and this material (combined with in-stream sources of detritus) may drive increased production among detritivorous stream insect larvae (Minshall 2003). However, a parallel study to this one, located on the same study streams, compared flux of allochthonous inputs between unburned and high severity burn categories and found that the high severity burn category had lower inputs of both deciduous and coniferous litter (B. Jackson and J. Braatne, College of Natural Resources, P.O. Box 441142, Moscow, ID 83844, USA, unpublished data). It is possible that there may be a difference in the nutrient quality of those allochthonous inputs, but this has not been investigated.

One further mechanism could be that rather than being caused by a change in basal resources, the observed differences in insect emergence may have been driven by disturbance-induced compositional differences in the invertebrate communities among sites. Previous studies have shown that benthic invertebrate communities can change in composition following wildfire (Minshall et al. 1997; Minshall et al. 2001). Generalist herbivore–detritivore taxa, e.g., *Baetis* (Baetidae) and Chironomidae, have fast life histories and seem to be well adapted for the variable physical conditions found in many postfire streams (Mihuc and Minshall 1995). As these *r* strategists (sensu Pianka 1970) produce more generations per year, a greater flux of emergence could be produced from sites that experienced high severity fire because of the faster turnover of these taxa. In fact, we did observe a greater flux of both Chironomidae and *Baetis* emerging from high severity burned sites than from either unburned or low severity burned sites (Malison 2008). Thus, the difference in emergence could have been driven by a shift in community composition to include more productive taxa following wildfire, serving to stimulate the flux of adult insect emergence to terrestrial habitats. Because of logistical and even ethical constraints, studies of wildfire effects are often based on comparative designs like this one, but determining the strength of effects and isolating mechanisms may require the control and replication of ambitious, large-scale field experimentation.

In addition to describing a midterm response to high severity fire, our results also suggest that different types of fire may have different impacts on stream-riparian ecosystems. Some of the strongest differences that we observed were between the high severity and low severity burned reaches. High severity fire appeared to stimulate the production of more benthic invertebrates, greater emergence, and more spiders and bats, whereas low severity fire seemed to have no effect on, or possibly even inhibited, these responses (low severity burned reaches had slightly lower values for some response variables than unburned reaches). Thus, it may be that the wildfire severity determines both the strength and direction of biotic responses. However, we do not know the mechanisms behind the differences that we observed between sites that experienced high versus low severity fire. The differences might simply have been the result of more sunlight entering the sites that experienced high severity versus low severity fire. Additionally, the sites that experienced low severity fire had a dense growth of riparian shrubs (reflected in greater shading and lower PAR than at unburned sites), even though their upper canopies were slightly more open than unburned reaches. Yet, if differences in insect productivity were only the indirect result of increased sunlight, we might have expected to detect differences in periphyton biomass. It is also possible that the differences between the high and low severity burn categories were, in part, due to an effect of stream size, as the low severity burn category included slightly smaller streams. Alternatively, it is possible that sites burned with high severity experienced hydrologic changes and associated streambed disturbance sufficient to shift the stream insect community to dominance by *r*-strategist taxa that resulted in greater emergence, whereas low severity fire did not trigger enough streambed disturbance to create such a community shift. Monitoring of some sites in the Big Creek watershed shows that a major scouring event had not occurred between the 2000 fire and the year of this study (G.W. Minshall, Idaho State University, Department of Biological Sciences, Pocatello, ID 83201, USA, unpublished data). Thus, there may be a threshold of disturbance frequency or intensity (Townsend 1989) that may explain differences between sites experiencing high versus low severity fire. To address this possibility, more studies comparing the effects of wildfire of varying severity on stream-riparian ecosystems are necessary, as are investigations that address the roles of climate, geomorphology, and hydrology as potential mediators of watershed responses to fire.

Inference and application of the results of this study may depend on both scale and land use context, the effects of which are unknown. Our results suggest that high severity fires do not play the same ecological role as low severity fires and allowing high severity fires to burn (rather than suppressing them) in certain forest types could be important in maintaining ecosystem function (Hutto 2008). We observed these patterns as a local effect at the scale of stream reaches, but responses to wildfire may differ at larger scales. For instance, in the western United States, wildfires are now occurring at higher magnitude and frequency (Westerling et al. 2006) such that entire river basins (e.g., Salmon River, Idaho) have been dominated by stand-replacing fires over the past 20 years. In such cases, effects at large spatial and longer time scales might include overall drying, with consequences for stream-riparian ecosystems that may contrast with those reported here. In addition, we conducted this study in a wilderness area where streams have been less impacted by humans and the linkages between streams and their surrounding riparian zones are essentially intact. The effects of wildfire might be much different for stream-riparian areas that have been altered or where linkages have been disconnected. The results of this study support the idea that high severity wildfire may amplify reciprocal fluxes between water and land for many years after the event, but future investigations are needed to address the effects in a land use context and through questions of scaling to build a deeper understanding of the role of wildfire in watershed ecosystems.

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Experimental assessment of the bioenergetic and behavioural differences between two morphologically distinct populations of Arctic char (*Salvelinus alpinus*)

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Abstract: A common environment experiment was conducted to assess the magnitude of the difference in growth, consumption, activity rate, and spatial and temporal patterns of habitat use between morphologically different populations of Arctic char (*Salvelinus alpinus*) originating from two Norwegian lakes. These two lakes contrasted sharply in terms of surface area, depth, elevation, length of the winter period, and fish community structure (presence–absence of brown trout, *Salmo trutta*). The experimental framework encompassed four littoral enclosures (average volume, 146 m^3) stocked with char from either of the two populations with duplicated treatments. Char morphology was quantified with numerical image analysis, food consumption was estimated using caesium analysis (^{133}Cs), and activity cost and patterns were determined using video cameras. Char populations were morphologically distinct and reacted differently in growth (1.9-fold difference between populations), food consumption (3-fold difference), and spatial activity patterns (20-fold difference) to the conditions prevailing in the enclosures. The results highlight that functional differences between morphologically distinct char may drive important differences in their bioenergetic and behavioural responses when exposed to similar environmental conditions. Such functional differences should be incorporated when developing habitat or trophic cascade models.

Résumé : Une expérience en conditions communes a été réalisée afin de quantifier la magnitude des différences de croissance, de consommation, d'activité et de patrons spatiaux et temporels d'utilisation d'habitat chez des ombles chevaliers (*Salvelinus alpinus*) provenant de deux populations morphologiquement différentes, issues de deux lacs de Norvège. Ces deux lacs différaient de manière marquée en terme d'aire, de profondeur, d'altitude, de la durée de leur période d'hivernage et de structure de leurs communautés de poissons (présence ou absence de truite brune, *Salmo trutta*). Le cadre expérimental utilisé comprenait quatre enclos de grandes tailles (volume moyen, 146 m^3), chacun ensemencé avec des ombles provenant de l'une ou l'autre des populations. Les réponses bioénergétiques ont été estimées en termes de taux de croissance, de consommation et d'activité et des patrons spatiaux et temporels d'activité. Nous avons quantifié la morphologie des ombles, estimé leur taux de consommation à l'aide d'un traceur chimique (^{133}Cs) et estimé leur taux d'activité et leurs patrons d'activité à l'aide de vidéo-caméras. Les deux populations d'omble chevalier sont morphologiquement distinctes et ont réagi différemment en termes de taux de croissance (un différence d'un facteur de 1,9 entre populations), de consommation (différence d'un facteur de 3) et de patrons spatiaux d'activité (différence d'un facteur de 20) aux conditions qui prévalaient dans les enclos. Ces résultats suggèrent que les différences fonctionnelles entre des morphs d'ombles chevaliers peuvent engendrer des différences importantes dans la façon avec laquelle ils répondent, d'un point de vue bioénergétique et comportemental, à un ensemble de conditions environnementales semblables. Étant donné leurs grandeurs, ces différences devraient être considérées lors de la construction et de l'application de modèles de qualité d'habitat ou de modèles de cascade trophique.

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Introduction

Niche adaptation is a fundamental issue in ecology. Niche adaptation may occur when organisms improve their ability to exploit their habitat through phenotypic plasticity and natural selection. In cases in which organisms respond to distinct environmental niches such as epibenthic and pelagic habitats, this response may result in polymorphism (Jonsson and Jonsson 1993; Sol et al. 2005). Groups of fish of a given species that differ morphologically are called morphs, notwithstanding the origin of this difference (in response to physical conditions or biological processes) or the existence of genetic differences between the groups. Morphs can exploit their environment differently (Skúlason and Smith 1995; Smith and Skúlason 1996; Losos 2000). Polymorphism occurs within most vertebrate groups such as fishes (Jonsson et al. 1988; Robinson and Wilson 1994; Keeley et al. 2005), amphibians (Maerz et al. 2006; Pfennig et al. 2006), reptiles (Hatase et al. 2007), and birds (Galeotti et al. 2003). Morphs can be so different that they initially were classified as separate species. For instance, morphs of Arctic char coexisting in different lakes or different habitats of the same lake were initially described as different species before being classified as variants of Arctic char (*Salvelinus alpinus*; Behnke 1972; Nordeng 1983; Jonsson and Jonsson 2001). In this species, morphs inhabiting the same lake have been described on the basis of differences in morphology and habitat use as epibenthic and pelagic morphs (Hindar and Jonsson 1982, 1993; Jonsson et al. 1988). A similar within-lake dichotomy has been described in brook trout (*Salvelinus fontinalis*; Bourke et al. 1997; Dynes et al. 1999). These differences in morphology and habitat use have several implications in the study of evolutionary and ecological processes.

Polymorphism is presumed to represent adaptive responses to attributes found in the local environment. These attributes may encompass physical conditions such as thermal regime (Larsson et al. 2005) and habitat structure (Robinson et al. 1996; Imre et al. 2002; Peres-Neto and Magnan 2004) and biological interactions such as competition and predation (Skúlason and Smith 1995; Robinson et al. 2000; Pfennig et al. 2007). Polymorphism has been the focus of numerous studies in the past three decades because of its potential importance in explaining evolutionary processes (e.g., sympatric speciation, adaptive radiation; Sage and Selander 1975; Smith and Skúlason 1996; Gíslason et al. 1999) and, more recently, because of its potential impact on species-oriented wildlife conservation practises (Jonsson and Jonsson 2001; Adams et al. 2007). Both the fundamental and practical implications of polymorphism depend on the functional differences among morphs. Such functional differences may include variation in life history traits (e.g., asymptotic length, size at sexual maturity, fecundity), as has been observed between morphs of Arctic char inhabiting postglacial lakes and northern rivers (Jonsson and Jonsson 2001). There may also be differences in foraging activity and rates of attack at specific depths, as has been shown for pelagic and littoral morphs of brook trout (Marchand et al. 2003). Furthermore, previous studies have examined the performance, in terms of growth and attack rate on food items, of different fish morphs in controlled experiments (Schluter and McPhail 1992; Schluter 1994; Andersson 2003). However, to our knowledge, no study has simultaneously quantified the functional differences between morphs of a species using a more complete range of key bioenergetic components (i.e., growth, food consumption, and activity cost) and using behavioural attributes that refer to spatiotemporal aspects of morph–environment interactions (i.e., spatial and temporal patterns of habitat use). Such elements may further define the relative performance of morphs (fitness index) under specified environmental conditions and the impacts that they may have on prey communities.

Our general objective was to assess key functional differences in bioenergetics and behaviour that may exist between morphs. This requires that morphs are identified and that variables describing functional responses are estimated under similar environmental conditions. Arctic char may be particularly useful in this regard because it has long been recognized to possess many morphs (e.g., Hindar and Jonsson 1982; Snorrason et al. 1994; Jonsson and Jonsson 2001) and is renowned for its great flexibility at using different, often contrasting, habitats (Klemetsen et al. 2003). In this context, our specific objectives were to (*i*) assess whether Arctic char from two populations inhabiting contrasting lakes (different in terms of surface area, depth, elevation, length of the winter period, and fish community structure) represent different morphs, and (*ii*) quantify differences in growth, consumption, and activity rates, as well as spatiotemporal patterns of habitat use, of these fish under similar environmental conditions.

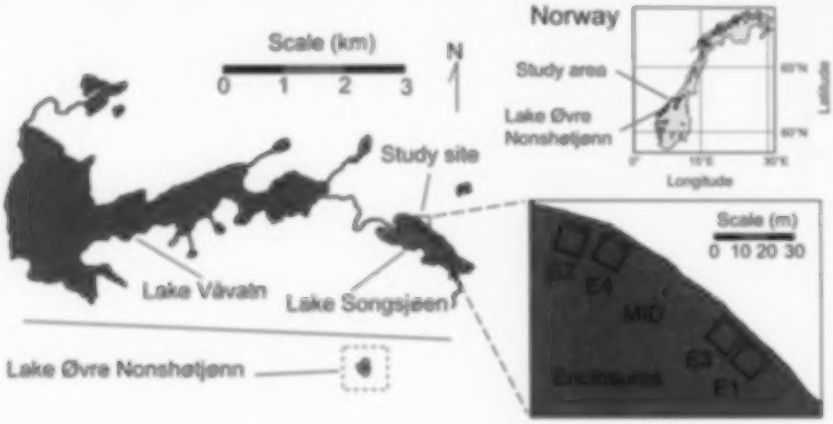
Materials and methods

The magnitude of between-population differences in bioenergetic and behavioural responses was investigated by comparing estimates of growth, consumption, and activity rates of Arctic char, as well as their spatial and temporal patterns of habitat use. The fish originated from two contrasting ecosystems and were exposed to similar environmental conditions in experimental enclosures. At the end of the experiment, we also measured a set of morphological traits on each char to establish whether they differed between the char populations. Estimates of consumption rates were obtained using chemical tracer (caesium; Forseth et al. 1992), whereas activity rate and spatiotemporal patterns of habitat use were obtained using video cameras. Observations were performed on individual fish to quantify morphology, growth, and consumption rate and by filming groups of fish in experimental enclosures to estimate activity rate and habitat use.

Site and enclosures

The experiment was conducted during summer 2001 in Lake Songsjøen, western Norway (63°19′26″N, 9°39′55″E; elevation = 262 m above sea level; area = 70 ha; Fig. 1). Lake Songsjøen is oligotrophic and supports populations of Arctic char and brown trout. Neither of the char populations under study had previous experience with the conditions prevailing in this lake. During 28 May – 3 June, four experimental enclosures (9.5 m × 9.5 m surface area, denoted E1 to E4) were deployed 3–5 m from the shoreline. Each enclosure consisted of a net (7 mm bar mesh size) attached to a frame and was positioned to minimize among-enclosure var-

Fig. 1. Study area (Songli, Norway), Lake Øvre Nonshøtjønn, study site, and position of the four experimental enclosures (E1–E4) deployed during summer 2001.



iation in average depth and water volume. Depth at the shallow end of the enclosures ranged from 75 to 101 cm (average = 86 cm), and depth at the deep end of the enclosures ranged from 232 to 251 cm (average = 238 cm). The volume of the enclosures ranged from 143 to 151 m^3 (average = 146 m^3). To prevent fish from escaping, the netting of the enclosures was sunk into the sediments or secured with gravel bags over hard substrate along the lake bottom and extended to 75 cm above the water surface. The absence of surface or bottom netting allowed the char to feed on prey throughout the water column, at the water surface, along the lake bottom, and within the sediments.

Experimental fish

The two char populations used for the experiment were taken from Lakes Våvatn (63°19′41″N, 9°34′29″E, 1.2 km upstream from Lake Songsjøen; elevation = 298 m above sea level; Secchi depth = 6–8 m; Fig. 1) and Øvre Nonshøtjønn (62°43′05″N, 9°32′03″E, 68 km south of Lake Songsjøen; elevation = 1004 m above sea level; Secchi depth = 10 m). In a previous study, neither population showed significant within-lake differences in morphology between benthic and pelagic habitat nor any significant relations between morphology and diets (Homme 2007). Thus, we regarded each of these populations as being composed of a single morph.

Lake Våvatn has an area of 425 ha and a maximum depth of 70 m and is covered by ice from mid-November to mid-May. The char population inhabiting Lake Våvatn live in sympatry with brown trout and feed predominantly on zooplankton (Langeland et al. 1991; L'Abée-Lund et al. 1993). Their mean total length is 18.6 cm (range = 9–28 cm; n = 1517 fish; Langeland et al. 1991), and their growth could be described by a von Bertalanffy model with an asymptotic length (L_∞) of 25.5 cm and a Brody growth coefficient (K) of 0.273 $years^{-1}$ (Vøllestad and L'Abée-Lund 1994). Because Lake Våvatn is located above the postglacial marine limit (150–200 m above present day sea level in this part of Norway), char and trout inhabiting this lake were most probably stocked by humans (Huitfeldt-Kaas 1918; Hesthagen and Sandlund 1995; Tammi et al. 2003). However, historical accounts as to when stocking might have occurred are lacking.

The second population originated from Lake Øvre Nonshøtjønn, which is small (area = 3.5 ha) and shallow (maximum depth = 17 m) and is covered by ice from the end of October to mid-June. The char population inhabiting Lake Øvre Nonshøtjønn is allopatric and has a diet mainly composed of surface insects (37%, percentages by volume), aquatic dipterans (26%, mostly pupae of Chironomids), and planktonic crustaceans (25%; Homme 2007). Their mean total length is 18.5 cm (range = 9–25 cm; n = 147 fish; collected in 1999 and 2000 using gillnets with similar series as in Langeland et al. 1991), and their growth could be described by a von Bertalanffy model with an L_∞ of 22.8 cm and a K of 0.553 $years^{-1}$. This population was introduced during 1905–1915 (exact date not known; Homme 2007). After conducting the present experiment, we became aware of the fact that brown trout (total n = 115) were stocked in Lake Øvre Nonshøtjønn in the period 1996–1999 (Øyvind Solem, Norwegian University of Science and Technology, Trondheim, personal communication). Gillnets set in August 2001 captured 108 char and no trout (Homme 2007), suggesting that brown trout stockings were unsuccessful. We therefore regarded the impact of the stocked trout on the char population of Lake Øvre Nonshøtjønn as negligible.

During 25 May to 15 June 2001, 41 juvenile char (ages 2+ and 3+; average size = 24.4 g (range = 13.0–55.5 g)) were collected from Lake Våvatn and 42 juvenile char (ages 2+ and 3+; average size = 37.3 g (range = 10.2–93.5)) from Lake Øvre Nonshøtjønn. Char from both lakes were collected using baited minnow traps and kept in separate cages (several cages per population) located in a pool of a tributary of Lake Songsjøen until the onset of the experiment. On 20 June 2001, fish were anaesthetized using clove oil (25 $mL \cdot L^{-1}$), weighed (±0.1 g; electronic balance model BB1200, Mettler-Toledo Inc., Mississauga, Ontario, Canada), and marked individually using alcian blue dye (blue 8GX, Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada). Handling time was less than 90 s per fish and the fish were allowed to recover for at least 15 min in aerated water.

Char were returned to the cages before being stocked into experimental enclosures.

Experimental procedure

The growth, consumption, and activity rates, as well as the spatiotemporal patterns of habitat use of char were estimated for duplicated treatments (i.e., char from Lake Våvatn, enclosures E1–E2; char from Lake Øvre Nonshøtjønn, enclosures E3–E4). The duration of the experiment was set to 43–44 days to have sufficient time for char growth, caesium accumulation (required for the estimation of consumption rate), and filming. Prior to the experiment, baited hook lines (two lines with four hooks) were set for 24 h in each enclosure and followed up by snorkel inspections to insure that the enclosures contained no fish. On 20 June 2001, 60 char (15 char per enclosure) were distributed among the enclosures to ensure similar among-enclosure means and variances in body mass (Table 1). The char density in the enclosures (16.6 fish·100 m^{-2}) was chosen to reduce the filming time required to estimate activity rate but was above the range of densities known from the literature for Scandinavian lakes (4.6–11 fish·100 m^{-2}; Langeland 1986; Byström et al. 2004). The 23 char not stocked into the enclosures (11 from Lake Våvatn and 12 from Lake Øvre Nonshøtjønn) were killed to obtain a baseline estimate of ^{133}Cs concentration in char at the beginning of the experiment (see Caesium analysis and computation of consumption rate).

Zooplankton sampling occurred on three occasions (13 June and 11 and 27 July) using a 5 L sampler, and samples were sieved through a 95 μm Nytex sieve. On each occasion, three samples, each obtained by combining four 5 L subsamples (total volume, 20 L), were collected at different locations in the study site (site depth, 2.0 ± 0.1 m). Two of these samples were collected within pairs of adjacent enclosures (denoted E1–E3 and E2–E4; two 5 L subsamples per enclosures × two enclosures), whereas a third sample (four 5 L subsamples) was collected outside the enclosures, mid-distance between enclosures E3 and E4 (denoted MID; Fig. 1). Zoobenthos was sampled shortly before (18 June) and after (5 August) the experiment using an Ekman bottom sampler (surface area = 225 cm^2). On each occasion, 10 samples were taken in each enclosures. Within 2 h of collection, samples were sieved through a 0.5 mm net and preserved in 95% ethanol.

The experiment was terminated over 2 days because of the time required to recapture and process the fish (E1 and E3 were terminated on 2 August; E2 and E4 were terminated on 3 August). Fish were recaptured by hauling a seine three to five times within each enclosure and were killed immediately by an overdose of anaesthetic. They were identified individually, weighed, photographed (Fleming et al. 1994; Fleming and Einum 1997; camera model DCR-VX 1000E, Sony of Canada Ltd., Toronto, Ontario, Canada), and dissected. The stomach contents of char taken from a given enclosure were pooled to obtain sufficiently large samples for ^{133}Cs analysis. All tissues and stomach contents were kept frozen (–80 °C) for ^{133}Cs analysis.

Video sampling

The activity rate of char was estimated from their swimming behaviour according to Boisclair (1992). Fish swimming behaviour was recorded using stereo video cameras (SVCs), which consisted of a pair of cameras (either model WV-BL602 or model WV-BP312, Panasonic Canada Inc., Mississauga, Ontario, Canada) enclosed side by side in a water-resistant (Plexiglas) case, 10 cm from one another. Two SVCs were used, allowing simultaneous observation at two locations of the enclosure. The four images produced by the two SVCs were assembled into a single image using a Quad image processor (model WJ-410, Panasonic Canada Inc.). A time–date generator (model WJ-810, Panasonic Canada Inc.) was used to add the time of filming to the images (±0.001 s). The resulting video sequences were recorded using a VHS videocassette recorder (model AG-1330, Panasonic Canada Inc.).

The spatial distribution of fish was quantified by defining four sampling zones (denoted A, B, C, and D; Fig. 2). Zones A and B included the entire water column within the shallowest and second shallowest thirds of the enclosure surface, respectively. Zones C and D included the remaining deepest third of the enclosure, with zone C comprising the volume below the surface to a depth of 110 cm and zone D, the remaining volume below 110 cm. The average volumes of zones A, B, C, and D were 31, 50, 33, and 33 m^3, respectively. SVCs were positioned such that zones A or B were filmed simultaneously with zones C or D. The SVC filming zones of A and B were positioned 50 cm from the perpendicular sides of the enclosures (perpendicular to shore), 75 cm below the water surface (35–75 cm above the bottom), and 3.15 m offshore from the nearshore enclosure wall (between zone A and B). This SVC was oriented parallel to water surface, and its yaw angle (relative to the enclosure wall) was changed to film zone A (45° to 60° toward shore) or zone B (75° to 85° toward offshore). The SVC sampling zones C and D was positioned 50 cm from the offshore enclosure wall, 110 cm below the surface (120–140 cm above the bottom), with its yaw angle oriented perpendicular with the wall. The pitch angle of the cameras (relative to the surface) was set 20°–30° upward when sampling zone C and 10° downward when sampling zone D. The total duration of a filming session was 1 h: two zones were filmed for 30 min (A and C, for example), then the orientation of the SVC was changed and the remaining zones (B and D in this example) were filmed for 30 min. The SVC took 10–20 min to reposition, and an additional 5 min was allowed before recording was resumed (see Appendix A for video analysis and computations).

Fish behaviour in each enclosure was quantified during two days. Filming began 14 days after the onset of the experiment to allow fish to acclimatize. Fish from each enclosure were filmed a first time over a period of eight days (E3, 4 July; E4, 7 July; E2, 9 July; and E1, 11 July) and a second time 16–22 days later over a period of seven days (E3, 23 July; E2, 25 July; E1, 27 July; and E4, 29 July). On any given sampling day, filming was organized into four sessions beginning at 0400, 1000, 1600, and 2200 and denoted 04 h (early morning), 10 h (late morning), 16 h (late afternoon), and 22 h (evening), respectively. However, to obtain good recordings as day length decreased, the starts of the filming sessions were progressively shifted to 0630, 1030,

Table 1. Variables estimated in the experimental enclosures and results of statistical tests of among-enclosure differences.

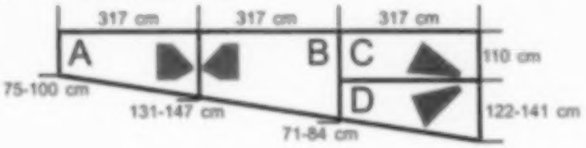
			Enclosure				Among-enclosure differences		
Variable	Mean	Range	E1	E2	E3	E4	$F_{3,v}$	v	p
Mass (g fresh weight)									
Initial	22.45 [21.18, 23.79]	10.2, 55.2	21.43 [19.04, 24.12]	21.24 [18.87, 23.91]	24.02 [21.34, 27.04]	23.21 [20.62, 26.12]	0.2597	56	0.85
Final	32.50±1.90	15.2, 65.0	24.85±3.65	30.41±3.81	37.73±3.51	36.11±3.51	2.656	45	0.06
No. of fish recaptured	12	11, 13	12	11	13	13	—	—	—
Fish density (fish·100 m^{-1})									
Initial	10.26	9.95, 10.52	9.95	10.52	10.39	10.20	—	—	—
Final	8.38	7.71, 9.00	7.96	7.71	9.00	8.84	—	—	—
Biomass density (g·m^{-1})									
Initial	2.30	2.13, 2.50	2.13	2.23	2.50	2.37	—	—	—
Final	2.73	1.98, 3.40	1.98	2.35	3.40	3.19	—	—	—
Growth rate (kJ·day^{-1})	1.00±0.08	–0.32, 2.44	0.52a±0.14	0.86ab±0.16	1.42b±0.14	1.14b±0.14	6.167*	43	0.001
Cs burden (ng)									
Initial	519 [482, 559]	253, 1440	385 [341, 435]	369 [323, 422]	681 [606, 766]	675 [600, 759]	—	—	—
Final	964 [864, 1075]	311, 4129	496a [439, 560]	478a [418, 547]	1710b [1520, 1924]	1722b [1530, 1937]	34.923	44	<0.001
Consumption rate (kJ·day^{-1})	5.22 [4.70, 5.79]	1.47, 21.52	2.90a [2.53, 3.33]	2.82a [2.43, 3.28]	8.73b [7.66, 9.95]	8.60b [7.55, 9.81]	21.825*	43	<0.001
Swimming speed (cm·s^{-1})	13.59±0.51	3.6, 25.0	10.76a±0.90	10.72a±0.82	15.46b±0.72	16.92b±0.90	14.197	72	<0.001
Activity rate (kJ·kg^{-1}·h^{-1})	1.37 [1.19, 1.59]	0, 13.68	1.14ab [0.84, 1.54]	1.54ab [1.19, 2.00]	2.08a [1.66, 2.60]	0.74b [0.51, 1.08]	3.2028†	100	0.05

Note: Mean values of the variables are presented with their standard error (mean ± 1 SE) or in the form "mean [mean – 1 SE, mean + 1 SE]" for asymmetric intervals. Enclosures of equal means are labelled using a and b following the mean. Enclosures labelled as "ab" are not statistically different from both a and b.

*After correcting for the effect of body mass.

†After correcting for the effect of sampling zone and time of day.

Fig. 2. Lateral cut-out view of an experimental enclosure with the delineated sampling zones used to estimate activity rate, their dimensions, and the positions of the volumes sampled by the stereo video cameras (shaded areas).



1530, and 2030 from 23 July and onward. Following this schedule, 32 h of filming were obtained (4 sampling sessions × 2 sampling sessions per enclosure × 4 enclosures). As four sampling zones were filmed, the total number of zone-specific estimates of activity rate was 128.

Zooplankton and zoobenthos analyses

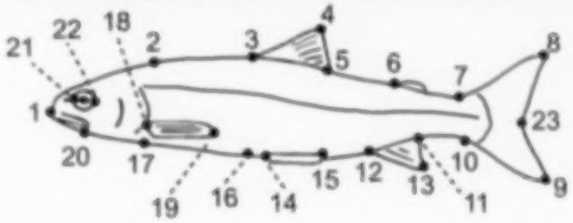
Zooplankton from the samples collected before, during, and after the experiment were identified to genera (cladocerans (*Holopedium*, *Bosmina*, *Sida*, *Diaphanasoma*, *Polyphemus*, and *Leptodora*); copepods, adults and copepodites (*Cyclops*, *Diaptomus*, and *Heterocope*)), counted, and measured under a dissecting microscope. Body length was measured on whole animals and was converted to dry mass using length–mass regressions (Breistein and Nøst 1997). Volumetric biomass data (grams dry weight $(\mathrm{dw})\cdot\mathrm{m}^{-3}$) were $\log(x + 1)$-transformed before statistical analysis.

Zoobenthos sampled at the beginning and end of the experiment were identified and counted under a dissecting microscope and grouped into four classes: benthic insects (larvae of Ephemeroptera, Plecoptera, Trichoptera, Odonata, Zygoptera, and Coleoptera), dipteran larvae (families Chironomidae and Ceratopogonidae), mollusks (snails and mussels), and others (Nematoda, Hirudinea, and Oligochaeta). Zoobenthos data were expressed in terms of number of animals per square metre.

Morphological analysis

Fish morphology was quantified using the photographs (numerical bitmaps; resolution: 720 × 576 pixels) taken at the end of the experiment. Morphological measurements involved three steps. First, a set of 23 landmarks (Fig. 3) was plotted on each image and landmark coordinates (x, y; in pixels) were measured using an image analysis software (ImageJ version 1.25I, rsbweb.nih.gov/ij/index.html). Second, a total of 29 morphological characters, each represented by virtual distances (in pixels) between two given landmarks, were calculated. Among these variables, five were used to describe the shape of the head (HEAD1–HEAD5), fifteen for the shape of the trunk (BODY01–BODY15), eight for the shape of the fins (FIN1–FIN8), and one for the diameter of the eye (EYE; Table 2). Third, the real values of the characters (millimetres) were calculated by multiplying their virtual values by a pixel conversion factor (millimetres per pixel), obtained for each picture using the image of a ruler (graduated to millimetres) that was photographed with the fish.

Fig. 3. Positions and descriptions of the 23 landmarks used for the analysis of morphological difference between the char populations.



Legend

1. Anterior end of the head
2. Dorsal-posterior end of the head
3. Origin of the dorsal fin
4. End of the dorsal fin
5. End of the dorsal fin insertion
6. Origin of the adipose fin
7. Dorsal origin of the caudal fin
8. Dorsal end of the caudal fin
9. Ventral end of the caudal fin
10. Ventral origin of the caudal fin
11. End of the anal fin insertion
12. Origin of the anal fin
13. End of the anal fin
14. Origin of the pelvic fin
15. End of the pelvic fin
16. Origin of pelvic cartilage
17. Ventral-posterior end of the head
18. Origin of the pectoral fin
19. End of the pectoral fin
20. Posterior end of the mouth
21. Eye perimeter: anterior measure
22. Eye perimeter: posterior measure
23. Caudal fork

Diet analysis

The diet of char was assessed by analyzing their stomach contents at the end of the experiment. Prey animals in the stomach contents were identified, counted, and measured under a dissecting microscope. Crustaceans were identified to genus, whereas other animals were identified to family or order. For larger animals, all individuals were counted and measured, whereas for crustaceans, representative subsamples were counted and measured. Body length was measured on whole animals or specific body parts. In the latter case, regression equations were used to estimate body length, which was converted to dry mass using length–mass regressions (Breistein and Nøst 1997). The diet of each fish was expressed as proportion of dry mass by prey type, and the average mass of the prey animals in the diet of each fish was calculated.

For analyses, the prey animals were grouped into seven categories: surface insects (terrestrial insects and adults of the aquatic Ephemeroptera, Trichoptera, and Diptera), benthic insects (see Zooplankton and zoobenthos analysis), mollusks (snails and mussels), aquatic dipterans (larvae and pupae of the families Chironomidae and Ceratopogonidae), planktonic cladocerans (species of genera *Daphnia*, *Bosmina*, *Holopedium*, *Polyphemus*, and *Bythotrephes*), planktonic copepods (*Heterocope* and *Cyclops*), and benthic crustaceans (*Eurycercus lamellatus* and *Sida crystallina*). Proportions of dry mass were arcsine-square-root-transformed before statistical analysis to normalize their probability distributions.

Caesium analysis

We estimated consumption rate using a chemical tracer (caesium) to avoid handling the fish during the experiment. Other methods (e.g., Eggers 1977; Elliott and Persson 1978) would have required that potentially fatal manipulations (sampling the stomach content of fish) be performed during the experiment. We considered that such manipulation

Table 2. Morphological variables, their associated landmarks, and allometry coefficients (and their R^2_{adj}) used to standardized the variables with respect to fork length, variable means and ranges estimated on individual Arctic char (*Salvelinus alpinus*), and formal descriptions.

	Landmarks		Allometry					
Variable	*a*	*b*	a_j	b_j	R^2_{adj}	Mean (mm)	Range (mm)	Description
L_F	1	23	—	—	—	150.04	122.2–191.8	Fork length
HEAD1	1	2	–0.858	0.817	0.798	25.44	19.9–33.8	Dorsal head length
HEAD2	1	20	–1.880	0.882	0.679	12.65	9.3–16.2	Mouth length
HEAD3	17	20	–1.662	0.911	0.833	18.26	14.8–23.8	Ventral head length behind mouth
HEAD4	2	20	–1.081	0.843	0.809	23.14	17.9–30.0	Transversal head length behind mouth
HEAD5	2	17	–1.039	0.836	0.937	23.38	19.1–29.1	Head depth
BODY01	2	3	–1.910	1.117	0.933	39.99	32.5–52.0	Anterior-dorsal length
BODY02	14	17	–1.539	1.045	0.941	40.42	31.6–52.5	Anterior-ventral length
BODY03*	3	6	–1.067	0.992	0.916	49.68	39.4–66.4	Median-dorsal length
BODY04	12	14	–1.747	1.038	0.905	31.66	25.5–41.5	Median-ventral length
BODY05	6	7	–2.130	1.002	0.757	18.03	13.8–23.4	Posterior-dorsal length
BODY06	10	12	–1.698	1.010	0.833	28.93	22.6–40.2	Posterior-ventral length
BODY07	2	14	–1.133	1.018	0.975	52.89	43.1–67.7	Anterior-transversal length 1
BODY08	3	17	–1.553	1.059	0.963	42.59	33.5–55.2	Anterior-transversal length 2
BODY09	3	16	–1.530	0.980	0.907	29.34	23.5–38.0	Body depth
BODY10	3	12	–1.475	1.059	0.966	46.15	36.4–60.4	Median-transversal length 1
BODY11	6	14	–1.124	1.000	0.955	48.86	39.5–61.9	Median-transversal length 2
BODY12	6	12	–2.212	1.073	0.921	23.69	18.1–31.6	Median-transversal depth
BODY13	6	10	–1.799	0.979	0.821	22.33	17.8–29.6	Posterior-transversal depth 1
BODY14	7	12	–1.615	1.029	0.938	34.45	27.8–45.4	Posterior-transversal depth 2
BODY15	7	10	–2.274	0.952	0.871	12.14	9.8–16.0	Caudal peduncle depth
FIN1	18	19	–1.716	0.968	0.892	22.93	18.8–31.7	Pectoral fin length
FIN2	3	4	–2.099	1.047	0.878	23.27	18.3–32.2	Dorsal fin leading edge length
FIN3*	12	13	–2.764	1.132	0.781	18.29	14.2–25.9	Anal fin leading edge length
FIN4	7	8	–1.985	1.082	0.854	31.13	23.6–42.8	Dorsal length of the caudal fin
FIN5	9	10	–1.460	0.991	0.821	33.32	26.0–44.7	Ventral length of the caudal fin
FIN6	3	5	–1.844	0.947	0.538	18.24	13.1–26.5	Length of the dorsal fin insertion
FIN7	11	12	–3.197	1.173	0.733	14.58	10.6–21.6	Length of the anal fin insertion
FIN8	14	15	–2.205	1.023	0.856	18.56	14.5–24.4	Length of the pelvic fin
EYE*	21	22	–1.366	0.692	0.412	8.16	6.2–10.3	Eye diameter

Note: Landmark numbers refer to those depicted in Fig. 3.

*Variables that significantly discriminated populations.

would have jeopardized the validity of the experiment. Caesium concentration (ng·g dw^{-1}) was estimated for individual char recaptured at the end of the experiment. Each fish was weighed (g fresh weight (fw)) and dried until mass stabilized (60 °C for 48–72 h) to determine its dry mass (g dw) and the proportion of dry matter. Dry fish tissues were then ground and a 300 mg subsample was taken and digested with HNO_3. Cs concentration (ng·g dw^{-1}) was measured on the digested subsample using a high resolution inductively coupled plasma mass spectrometer (ICP-MS; model Finnigan Element1, Thermo Electron Corp., Waltham, Mass.). Cs burden in char (ng fw) was obtained by multiplying the Cs concentration per gram of dry tissues by the proportion of dry matter and original fresh mass of the fish (g fw).

Computations

Growth rate

We calculated the growth rate (*G*, kJ·day^{-1}) as

$$(1) \qquad G = \frac{(w_F - w_0) \cdot \mathrm{ED}}{n}$$

where w_0 and w_F are the body mass of the fish (g fw) at the beginning and the end of the experiment, respectively; *n* is the duration of the experiment (days), and ED is fish energy density (kJ·g fw^{-1}) predicted by the model of Hartman and Brandt (1995, their eq. 13, developed for salmonids) using the proportion of dry matter.

Morphology

Morphological analysis was performed on size-corrected values of traits to compare fish on the basis of their shape rather than their actual size. The values (c_j) of any given morphological character *j* were size-corrected (the fork length was taken as an index of fish size) using the following equation (Reist 1986):

$$(2) \qquad c_j' = c_j \cdot \left(\frac{L_{\mathrm{STD}}}{L_F}\right)^{b_j}$$

where c_j' is the size-corrected value of the character, L_{STD} is the fork length used to standardize the characters (i.e., the mean length of all fish at the end of the experiment; mm), L_F is the fork length of the fish at the end of the experiment (mm), and b_j is the allometry coefficient between character *j* and the fork length of the fish (i.e., the slope of the relationship between log c_j and log L_F estimated for all fish; Table 2).

Consumption rate

Initial Cs burden of the fish was estimated from the Cs concentration of the 23 char sampled at the beginning of the experiment (Lake Vâvatn: average body mass = 28.18 g fw, range = 16.25–43.87 g fw; Lake Øvre Nonshøtjønn: average body mass = 63.91 g fw, range = 46.50–93.53 g fw). No statistically significant relationship was found between Cs concentration ($\log_{10}$-transformed) and the fresh body mass of char (Lake Vâvatn, $t_{[9]} = -0.427$, $p = 0.68$, $R^2_{adj} = -0.089$; Lake Øvre Nonshøtjønn, $t_{[10]} = 0.641$, $p = 0.5357$, $R^2_{adj} = -0.057$). As a consequence, the initial Cs burden (ng) of char was estimated by multiplying the mean initial Cs concentration (Lake Vâvatn, 19.56 ng·g fw^{-1}; Lake Øvre Nonshøtjønn, 28.57 ng·g fw^{-1}) by their fresh body mass at the start of the experiment (g fw). Cs concentration in the food consumed by char (pooled stomach contents) was evaluated using the same procedure as for body tissues and reported as the dry weight concentration (ng·g dw^{-1}).

Consumption rate was calculated for individual char following the method proposed by Forseth et al. (1992). For any given day i, we calculated the linearly interpolated body mass w_i (g fw) and Cs burden Q_i (ng) of the fish, and the Cs elimination rate (k_i, day^{-1}) was estimated from w_i and mean daily temperature (°C) using the model of Ugedal et al. (1992). Daily Cs intake (I_i, ng·day^{-1}) was calculated as proposed by Forseth et al. (1992, their eq. 3*b*), and mean daily consumption rate (C, kJ·day^{-1}) was calculated as

$$(3)\qquad C = \frac{\sum_{i=1}^{n} I_i \cdot \sum_{j=1}^{m} (p_j \cdot S_j)}{[\mathrm{Cs}]_f \cdot \mathrm{Eff} \cdot n}$$

where $[\mathrm{Cs}]_f$ (ng·g dw^{-1}) is the concentration of Cs in the diet of char from each enclosure, Eff is the Cs absorption coefficient (the proportion of the Cs found in the diet that is assimilated by the fish; Forseth et al. 1992), n is the duration of the experiment (days), p_j is the the proportion (calculated dry mass percentage) of prey item j in fish diet, and S_j (kJ·g dw^{-1}) is the energy density of prey item j (kJ·g dw^{-1}). The energy densities of prey items were obtained from Cummins and Wuycheck (1971). Where species-specific energy density was unavailable, generic values to the nearest taxonomic group were substituted. Because stomach contents were pooled, the energy density of the diet was estimated once for each enclosure.

Activity rate

Estimation of activity rate from swimming behaviour involved three steps (see Appendix A for a more detailed description of video analysis and computations). First, the biomass density ($B_{z,t}$, g fw·m^{-3}) of actively swimming char observed in a zone z and at time t in the volume sampled by the SVC ($V_{z,t}$, m^3) was calculated as

$$(4)\qquad B_{z,t} = \frac{\mathrm{FS}_{z,t}\overline{w}_t}{T_{z,t} \cdot V_{z,t}}$$

where $\mathrm{FS}_{z,t}$ is the total number of moving fish-seconds (fish·s^{-1}; see Appendix A). $\overline{w}_t$ is the mean body mass of fish inside the enclosure (g fw; linearly interpolated at the time of sampling), and $T_{z,t}$ is the duration of the recording (in seconds). Second, the mean swimming cost (MSC; kJ·kg^{-1}·h^{-1}) in zone z at time t was calculated using mean body mass and mean swimming speed ($\overline{v}_{z,t}$, cm·s^{-1}):

$$(5)\qquad \mathrm{MSC}_{z,t} = 13.54 \cdot 10^{b_0} \cdot \overline{w}_t^{(b_{\mathrm{MASS}}-1)} \cdot \overline{v}_{z,t}^{b_{\mathrm{SPEED}}}$$

where 13.54 is the oxycaloric coefficient (J·mg O_2^{-1}; Elliott and Davison 1975), and parameters b_0, b_{MASS}, and b_{SPEED} (±1 standard deviation) are, respectively, −1.411 ± 0.092, 0.915 ± 0.039, and 0.886 ± 0.096. The parameters b_0, b_{MASS}, and b_{SPEED} were obtained from the swimming cost model developed by Guénard et al. (2008). Third, activity rate ($A_{z,t}$, kJ·kg^{-1}·h^{-1}) in zone z at time t was calculated as

$$(6)\qquad A_{z,t} = \frac{B_{z,t} \cdot \mathrm{MSC}_{z,t}}{B_{e,t}}$$

where $B_{e,t}$ (g fw·m^{-3}) is the mean biomass density of fish in the enclosure per unit of volume (e.g., the sum of the interpolated body masses at time t divided by the volume of the enclosure).

Statistical analysis

Computations and statistical analyses were performed using R language and environment (version 2.4.1; R Development Core Team 2007). Statistical significance was examined by analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA). In all instances, analyses began with a test of among-enclosures differences, using the enclosures as a single factor, with four levels (the enclosures). When statistically significant, post hoc tests of differences between enclosures were done (Scheffé 1953). Then, tests of between population difference were performed with two factors, namely populations (enclosures with char from Lake Vâvatn or Lake Øvre Nonshøtjønn) and treatment replicates. In this design, factor treatment replicates is nested within factor populations. Calculation of F statistics was done using type III sums of squares whenever unbalanced designs occurred. Normality of the residuals was tested by the Shapiro–Wilk's test of normality (Shapiro and Wilk 1965). When residuals were found to be non-normally distributed, the explanatory variable was transformed using the Box and Cox (1964) method prior to analysis. Homogeneity of within-group or residual variance was tested using Bartlett's test (Snedecor and Cochran 1989) or by examining the plots of residual (y) and predicted (x) values. Morphological differences between char populations were assessed using forward-stepwise multiple logistic regressions (McCullagh and Nelder 1989). Analyses of activity rate and active biomass density were done using generalized linear models (McCullagh and Nelder 1989; quasi-Poisson likelihood model using logarithmic link function (Hastie and Pregibon 1992)). Statistical significance of the resulting deviance tables was calculated using an F test. For all statistical analyses, a significance threshold of 5% was used for type I (α) error. Type I error rates associated with multiple statistical inferences (e.g., forward-stepwise selection of morphological variables) were corrected sequentially using the Šidák in-

Table 3. (*a*) Biomass (g dw·m^{-3}) of the zooplankton genera found in the nine mixed samples performed on the study site, and (*b*) density of zoobenthos (no.·m^{-2}) in the experimental enclosures at the beginning and end of the experiment.

(*a*) Biomass of the zooplankton genera.

		13 June			11 July			27 July		
Order	Genus	E1–E3	MID	E2–E4	E1–E3	MID	E2–E4	E1–E3	MID	E2–E4
Cladocerans	*Holopedium*	0.04	0.00	0.00	2.51	2.77	7.01	0.00	0.00	0.10
	Bosmina	9.98	10.15	13.07	7.08	4.16	11.51	14.39	10.81	16.72
	Sida	0.00	1.85	1.33	0.00	0.00	1.02	0.06	0.21	0.88
	Diaphanosoma	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	Polyphemus	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Leptodora	0.00	0.00	0.00	0.00	0.00	25.0	0.00	0.21	0.00
Copepods	*Cyclops*	0.06	0.00	0.06	0.00	0.03	0.03	0.32	0.07	0.08
	Diaptomus	0.00	0.00	0.00	0.00	0.00	0.49	0.20	0.35	1.59
	Heterocope	4.90	0.44	0.88	20.8	8.75	6.17	0.81	0.70	0.08
Total		16.0	12.4	15.3	30.4	15.7	51.2	16.0	12.4	19.4

(*b*) Density of zoobenthos.

			Groups				
Time	Enclosures	*N*	Insects	Dipterans larvae	Mollusks	Other	Total
18 June	E1	10	8.9 [4.3, 18.3]	80.0 [56.4, 113]	97.8 [72.3, 132]	8.9 [3.6, 22.2]	187 [145, 241]
	E2	10	22.2 [14.1, 35.1]	66.7 [45.5, 97.8]	31.1 [18.2, 53.1]	17.8 [9.3, 34.0]	120 [87.4, 165]
	E3	10	22.2 [14.1, 35.1]	102 [75.0, 139]	107 [79.9, 142]	4.4 [1.2, 16.2]	231 [184, 290]
	E4	10	26.7 [17.6, 40.4]	142 [109, 185]	40.0 [25.0, 64.1]	26.7 [15.7, 45.2]	209 [164, 266]
5 August	E1	7	50.8 [35.4, 72.9]	0.0 [—,—]	114 [81.9, 159]	76.2 [52.4, 111]	165 [120, 228]
	E2	10	31.1 [21.2, 45.7]	107 [78.8, 144]	244 [202, 296]	4.4 [1.2, 16.2]	382 [320, 457]
	E3	7	6.4 [2.3, 17.6]	108 [75.3, 155]	95.2 [66.1, 137]	25.4 [13.3, 48.5]	210 [157, 279]
	E4	10	31.1 [21.2, 45.7]	129 [97.9, 170]	169 [134, 212]	0.0 [—,—]	329 [272, 398]
Grand mean		74	24.6 [20.8, 29.2]	94.9 [83.4, 108]	113 [100, 127]	18.0 [12.8, 25.3]	232 [213, 254]

Note: Format for density of zoobenthos in (*b*) is mean [mean – 1 SE, mean + 1 SE]. See main text for details on grouping of zoobenthos.

equality (Šidák 1967; Wright 1992). The corrected *p* values are reported as *p*′.

Results

Conditions in the enclosures

Water temperature varied between 13.5 °C and 19.6 °C over the course of the experiment (average = 16.3 °C). The total zooplankton biomass ranged between 12.4 and 51.2 g dw·m^{-3} (Table 3*a*; mean = 18.8 g dw·m^{-3}) and did not differ among the three sampling sites ($F_{[2,6]}$ = 1.5171, p = 0.29) and sampling times ($F_{[2,6]}$ = 3.0915, p = 0.12). No among-sites difference in the composition of the zooplankton community were found using a multiple analysis of variance performed on the five most abundant genera (i.e., *Holopedium*, *Bosmina*, *Sida*, *Leptodora*, and *Heterocope*; Wilk's $\lambda_{[2,6]}$ = 0.02926, p = 0.27). Among the zoobenthos samples taken at the end of the experiment, six were lost before counting the invertebrates, leaving seven replicates for enclosures E1 and E3. Mollusks and dipterans larvae (mainly chironomids) were the most abundant groups of benthic animals, accounting for 83% of the total density (Table 3*b*). The composition of the bottom fauna in the experimental enclosures was similar at the onset of the experiment ($\lambda_{[3,36]}$ = 0.789, p = 0.76) but differed at the end ($\lambda_{[3,30]}$ = 0.296, $p < 0.001$). The composition was affected by the char population ($\lambda_{[1,30]}$ = 0.592, p = 0.006), and differences were also detectable among treatment replicates ($\lambda_{[2,30]}$ = 0.439, p = 0.003). The most notable contrasts between char population treatments were found for dipteran larvae and benthic insect densities. Dipteran larvae were nearly twice as abundant in enclosures with char from Lake Øvre Nonshøtjønn (mean = 120 individuals·m^{-2}) than in those with char from Lake Våvatn (mean = 63 individuals·m^{-2}, $p < 0.001$). On the other hand, benthic insect density in enclosures with char from Lake Våvatn (mean = 39 individuals·m^{-2}) were twice as high as those with char from Lake Øvre Nonshøtjønn (mean = 21 individuals·m^{-2}, p = 0.05). Taken together, these results indicate that the taxonomic composition and the abundance of prey were relatively uniform among the enclosures at the beginning of the experiment but that the composition of zoobenthic prey differed at the end.

Recapture and growth

On average, 82% of the char were recaptured at the end of the experiment (range = 73% to 87%; Table 1). The growth rate of individual char was positively influenced by the body mass of char at the beginning of the experiment (b = 0.016 kJ·day^{-1}·g fw^{-1}, $F_{[1,43]}$ = 6.4441, p = 0.015, R^2_{adj} = 0.070), but the slope of the relationship between growth rate and initial body mass was similar among enclosures ($F_{[3,40]}$ = 1.7954, p = 0.16). Once corrected for body mass, growth rate differed significantly among enclosures (Table 1; Fig. 5*a*). Among-enclosure variation in growth rate was associated with differences between populations but not between treatment replicates (Table 4). Char originating from Lake Øvre Nonshøtjønn (mean = 1.28 kJ·day^{-1}·g fw^{-1}) achieved 1.9 times higher growth than char from Lake Våvatn (mean = 0.67 kJ·day^{-1}·g fw^{-1}).

Table 4. Statistical tests of the differences between Arctic char (*Salvelinus alpinus*) populations and between replicates of the population treatments.

	Difference among populations treatments and treatments replicates			
Variables	$F_{\nu 1,\nu 2}$	ν_1	ν_2	p
Growth rate*				
Population	12.6326	1	43	<0.001
Replicates	2.4494	2		0.10
Consumption rate*				
Population	65.1057	1	43	<0.001
Replicates	0.0067	2		0.99
Swimming speed				
Population	42.309	1	72	<0.001
Replicates	0.815	2		0.45
Activity rate†				
Population	0.0005	1	106	0.98
Replicates	3.9237	2		0.023
Population × zone	5.7055	3		0.001

*After correcting the effect of body mass.

†Models including the sampling zone and time of day.

Table 5. Diet composition (percent dry weight) and prey size (geometric mean milligrams dry weight) of Arctic char (*Salvelinus alpinus*) in the four experimental enclosures (E1–E4).

	Lake Våvatn		Øvre Nonshøtjønn	
Group	E1	E2	E3	E4
Surface insects	0.3 [0.1, 0.6]	0.8 [0.4, 1.2]	0.0 [0.0, 0.0]	0.8 [0.4, 1.2]
Benthic insects	0.0 [0.0, 0.1]	0.0 [0.0, 0.1]	0.5 [0.2, 0.8]	0.0 [0.0, 0.1]
Mollusks	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]
Aquatic dipterans	9.0 [6.0, 12.6]	19.9 [15.0, 25.2]	6.7 [4.2, 9.8]	5.1 [2.7, 8.1]
Planktonic cladocerans	84.6 [76.1, 91.5]	65.0 [53.4, 75.8]	10.4 [4.9, 17.5]	35.4 [25.1, 46.5]
Planktonic copepods	0.0 [0.0, 1.7]	1.9 [0.0, 7.0]	65.3 [54.1, 75.7]	47.7 [35.4, 60.0]
Benthic crustaceans	2.4 [1.1, 4.2]	2.1 [0.8, 3.9]	2.0 [0.9, 3.6]	0.5 [0.0, 1.5]
Mean prey size (mg)	0.010 [0.009, 0.012]	0.015 [0.012, 0.017]	0.037 [0.032, 0.043]	0.023 [0.020, 0.027]

Note: Format for diet composition and prey size: mean [mean – 1 SE, mean + 1 SE]. See main text for details on prey groups.

Morphology

The mean fork length of the char used for the experiment was 151.2 mm, and morphological traits were size-corrected to a standard value of fork length (L_{STD}) of 150 mm. The coefficients of determination (R^2_{adj}) of the relationships between the morphological traits and fork length (both natural-log-transformed) ranged from 0.412 to 0.975, and the allometry coefficient (*b*) ranged from 0.692 to 1.134 (Table 2). The char from the two populations differed in terms of eye diameter (EYE: $\chi^2_{[1]} = 14.259$, $p = 1.59 \times 10^{-4}$, $p' = 0.005$), anal fin length (FIN3: $\chi^2_{[1]} = 11.026$, $p = 0.001$, $p' = 0.03$), and distance between the anterior insertions of the main and adipose dorsal fins (BODY03: $\chi^2_{[1]} = 12.398$, $p < 0.001$, $p' = 0.007$; see Table 2 and Fig. 3 for morphological traits). The model including these three variables ($\chi^2_{[3]} = 38.683$, $p < 0.001$) could correctly classify 87.5% of the individual char to their original population. The linear estimator (λ) of the logistic model is

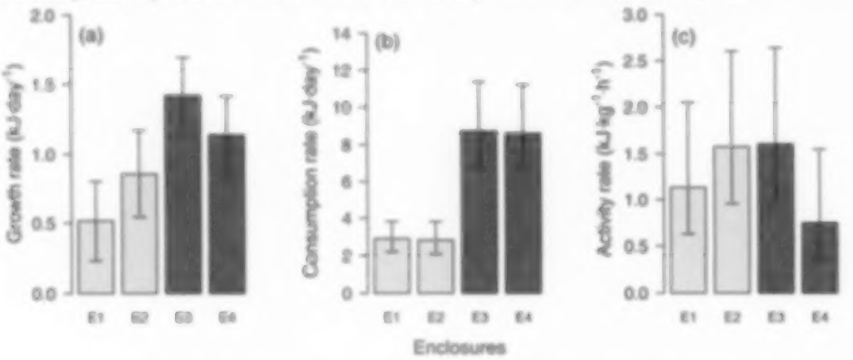
$$(7) \quad \lambda = -44.98 + 2.922 \cdot \mathrm{EYE}' - 1.919 \cdot \mathrm{FIN3}' + 1.118 \cdot \mathrm{BODY03}'$$

where variable names followed by a prime represent size-corrected traits values (mm). The linear predictor (λ) represents the degree of similarity to char from Lake Våvatn. Consequently, individual char having positive values of λ are predicted to originate from Lake Våvatn, and those having negative values are predicted to originate from Lake Øvre Nonshøtjønn. Char from Lake Våvatn were found to have 11% larger eye diameter (mean = 8.62 mm), 3.7% greater median dorsal length (mean = 50.7 mm), and 6.1% shorter anal fin (mean = 17.7 mm) than char from Lake Øvre Nonshøtjønn (means 7.79, 48.9, and 18.8 mm, respectively).

Diet composition

All fish stomachs contained food at the end of the experiment. Crustacean zooplankton (cladocerans and copepods) accounted for 85% of the diet of char, followed by aquatic dipterans (mean = 9.2%; Table 5). Diet composition differed among enclosures (Wilk's $\lambda_{[3,42]} = 0.2347$, $p < 0.001$), between char treatments ($\lambda_{[1,42]} = 0.4$, $p < 0.001$), and between treatment replicates ($\lambda_{[1,42]} = 0.6$, $p = 0.03$). The char from Lake Våvatn had proportionately 3.7 times more planktonic cladocerans in their diet (mean = 76.4%) than the char from

Fig. 4. Mean (±95% confidence limit, CL) (*a*) growth rate, (*b*) consumption rate (obtained from caesium), and (*c*) activity rate (obtained from video cameras) of Arctic char (*Salvelinus alpinus*) from two populations (Lake Våvatn, bars with light shading; Lake Øvre Nonshøtjønn, bars with darker shading) and exposed to similar conditions in four experimental enclosures (E1–E4).



Lake Øvre Nonshøtjønn (mean = 20.5%). Moreover, char from Lake Øvre Nonshøtjønn ate mostly planktonic copepods (mean = 57.3%), whereas this prey type accounted for only a small fraction of the diet of char from Lake Våvatn (mean = 0.5%). Char from Lake Våvatn had proportionately 2.3 times more aquatic dipterans in their diet (mean = 13.5%) than char from Lake Øvre Nonshøtjønn (mean = 5.6%; $F_{[1,44]} = 4.619$, $p = 0.04$). No differences were found among enclosures, between char treatments, and between treatment replicates when planktonic cladocerans, planktonic copepods, and aquatic dipterans were not included in the diet composition analysis, indicating that variation in diet composition was mostly associated with these three prey types. The mean prey size differed among enclosures ($F_{[3,42]} = 13.070$, $p < 0.001$), between char treatments ($F_{[1,42]} = 29.806$, $p < 0.001$), and between treatment replicates ($F_{[2,42]} = 3.3415$, $p = 0.045$). The prey consumed by char from Lake Øvre Nonshøtjønn were on average 2.5 times larger (mean = 0.030 mg) than that consumed by char from Lake Våvatn (mean = 0.012 mg).

Consumption rate

On average, the Cs body burden of char doubled during the course of the experiment and differed significantly among enclosures at the end of the experiment (Table 1). Char from Lake Øvre Nonshøtjønn contained 3.5 times more Cs at the end of the experiment (mean = 1716 ng) than char from Lake Våvatn (mean = 488 ng). Energy densities used to transform consumption rate from dry mass to energy were 21.93 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ for cladocerans, 24.22 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ for copepods, 22.69 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ (chironomids) for aquatic dipterans, 20.18 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ for surface insects, and 19.77 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ (mean value of arthropods) for all other prey items (Cummins and Wuycheck 1971). The estimated energy density of food ranged from 22.00 to 23.36 $\mathrm{kJ{\cdot}g\ dw^{-1}}$ (mean ± SD = 22.7 ± 0.67 $\mathrm{kJ{\cdot}g\ dw^{-1}}$). Cs concentration (mean ± SD) in the diet of char from Lake Våvatn was 55.3 ± 12.2 $\mathrm{ng{\cdot}g\ dw^{-1}}$ and 105.0 ± 5.3 $\mathrm{ng{\cdot}g\ dw^{-1}}$ for char from Lake Øvre Nonshøtjønn. Because zooplankton dominated the diet, consumption rates were calculated using the Cs absorption coefficient (0.816 ± 0.044) for zooplankton (Forseth et al. 1992).

Char consumption rate was positively influenced by initial body mass ($b = 0.31\ \mathrm{kJ{\cdot}day^{-1}{\cdot}g\ fw^{-1}}$, $F_{[1,46]} = 39.832$, $p < 0.001$), but the relationship between consumption rate and the initial body mass did not differ among enclosures ($F_{[3,40]} = 0.3732$, $p = 0.77$). When corrected for body mass, the consumption rate of char differed significantly among enclosures and between populations but did not differ between treatment replicates (Tables 1 and 4; Fig. 4*b*). Char from Lake Øvre Nonshøtjønn consumed three times more food (mean = 8.67 $\mathrm{kJ{\cdot}day^{-1}}$) than the char from Lake Våvatn (mean = 2.87 $\mathrm{kJ{\cdot}day^{-1}}$).

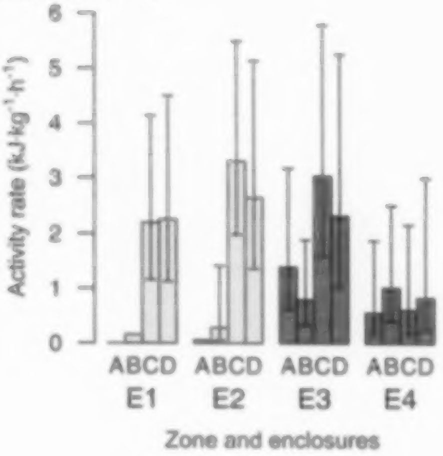
Activity rate and spatiotemporal patterns of habitat use

The water volume sampled by the SVC ranged from 0.099 $\mathrm{m^3}$ to 1.13 $\mathrm{m^3}$ (mean = 0.49 $\mathrm{m^3}$) depending on filming conditions. Among the 128 recordings collected, char were observed swimming in 91 recordings (76%), and it was possible to estimate swimming speed in 76 (59%) of them. Median fish-seconds (FS; $\mathrm{fish{\cdot}s^{-1}}$) observed during a 30 min recording was 14.0 $\mathrm{fish{\cdot}s^{-1}}$. Observed biomass density ($B_{z,t}$) ranged from 0 to 9.14 $\mathrm{g{\cdot}m^{-3}}$ (mean = 1.14 $\mathrm{g{\cdot}m^{-3}}$) among recordings, and mean biomass density ($B_{e,t}$) ranged from 2.26 to 3.69 $\mathrm{g{\cdot}m^{-3}}$ among enclosures. Mean swimming speed varied up to sevenfold among recordings, differed among enclosures, between char populations, but not between treatment replicates (Tables 1 and 4). Char from Lake Våvatn swam, on average, 50% faster (mean = 16.0 $\mathrm{cm{\cdot}s^{-1}}$) than char from Lake Øvre Nonshøtjønn (mean = 10.7 $\mathrm{cm{\cdot}s^{-1}}$).

Values of FS and activity rate ($\mathrm{kJ{\cdot}kg^{-1}{\cdot}h^{-1}}$) were estimated for each zone of the enclosures and for each time of day. Although FS corrected for the volume and the time spent filming ($\mathrm{FS{\cdot}m^{-3}{\cdot}h^{-1}}$) represents the time spent by fish in any given zone (notwithstanding their swimming speed during this time), activity rate represents the energy spent swimming by fish in these zones (combination of time spent swimming and swimming speed). In our study, both variables provided the same interpretation about habitat use in the enclosures. Hence, for practical reasons, only activity rates are presented to describe the spatiotemporal patterns of habitat use by fish.

The use by char of the different zones within the enclosures varied significantly ($F_{[3,124]} = 8.9071$, $p < 0.001$). Char were 3.9 times more active in zones C and D (mean =

Fig. 5. Mean (+ 95% confidence limit, CL) hourly activity rates of Arctic char (*Salvelinus alpinus*) char from two populations (Våvatn, bars with light shading; Øvre Nonshøtjønn, bars with darker shading) in four sampling locations (zones A, B, C, D) in four experimental enclosures (E1–E4).



2.16 kJ·kg^{-1}·h^{-1}) than in zones A and B (mean = 0.56 kJ·kg^{-1}·h^{-1}). However, differences between zones A and B and between zones C and D were small and not statistically significant (p = 0.83 and 0.74, respectively). Activity rate did not differ among times of the day ($F_{[3,112]}$ = 2.3950, p = 0.07), but the interaction between sampling zone and time of day was statistically significant (i.e., the spatial distribution of fish changed with time of day; $F_{[9,112]}$ = 2.1485, p = 0.03). During early and late morning (04 h and 10 h), char were 4.0–6.8 times more active in zone C (mean = 3.69–2.44 kJ·kg^{-1}·h^{-1}) than in the remaining zones (A, B, and D; mean = 0.55–0.61 kJ·kg^{-1}·h^{-1}), and a similar pattern was seen during the evening (22 h), though the difference was only 1.8 times (zone C, mean = 1.05 kJ·kg^{-1}·h^{-1}; zones A, B, and D, mean = 0.60 kJ·kg^{-1}·h^{-1}). During the afternoon (16 h), however, char were most active in zone D (mean = 5.35 kJ·kg^{-1}·h^{-1}), being 6.2 times more active than in other zones (A–C; mean = 0.86 kJ·kg^{-1}·h^{-1}).

Because these spatial and temporal patterns of activity encompass a significant fraction of fish activity and to increase statistical power, the factor sampling zone and the interaction between sampling zone and time of day were added to the analyses involving activity rate (e.g., among enclosure, between char treatments, and between treatment replicate differences in hourly activity rate). Hourly activity rate differed among enclosures (Table 1; Fig. 4) and in the manner by which char distributed their activity across space (i.e., the interaction between enclosure and sampling zone; $F_{[9,100]}$ = 2.6526, p = 0.008), indicating that the char maintained in different enclosures also had different spatial patterns of activity (Fig. 5). These differences in spatial patterns were associated with differences between char populations (Table 4). The tendency of char from Lake Våvatn to be more active in zones C and D than in zones A and B was 20 times more pronounced than that of char from Lake Øvre Nonshøtjønn. Char from Lake Våvatn were 35 times more active in zones C and D (mean = 2.60 kJ·kg^{-1}·h^{-1}) than in zones A and B (mean = 0.074 kJ·kg^{-1}·h^{-1}), whereas char from Lake Øvre Nonshøtjønn were only 1.7 times more active in zones C and D (mean = 1.59 kJ·kg^{-1}·h^{-1}) than in zones A and B (mean = 0.91 kJ·kg^{-1}·h^{-1}). Differences in spatial activity patterns between treatment replicates were not statistically significant ($F_{[6,88]}$ = 1.0558, p = 0.40). Moreover, activity rates were similar between char populations but differed between treatment replicates (Table 4), with char from Lake Øvre Nonshøtjønn in enclosure E3 being 2.1 times more active than in enclosure E4 (Table 1).

Discussion

The results of the morphological analysis indicate that the two populations studied represent different morphs of Arctic char. Char from Lakes Våvatn and Øvre Nonshøtjønn differed, particularly with respect to the diameter of the eye, the length of the dorsal-posterior area, and the length of the leading edge of the anal fin. Eye diameter may be related to acuity for detecting small prey under poor light conditions (i.e., Roo et al. 1999; Job and Bellwood 2000) such as those prevailing in the deep pelagic and profundal zones of lakes. Thus, larger eyes, exhibited by char from Våvatn, should improve the ability of fish to feed profitably on small prey by decreasing search time (i.e., Holling 1959). Char from Lake Våvatn also exhibited shorter anal fins and a greater median dorsal length than char from Lake Øvre Nonshøtjønn. These traits may be associated with reduced hydraulic drag that could diminish swimming cost (Boily and Magnan 2002; Ohlberger et al. 2006). The larger eye diameter and the more hydrodynamic shape of the char from Lake Våvatn compared with char from Lake Øvre Nonshøtjønn are consistent with the possibility that char from Lake Våvatn may represent a more pelagic morph than char of Lake Øvre Nonshøtjønn, which may, in turn, represent a relatively more littoral–benthic morph. The 50% faster mean swimming speed noted for char from Lake Våvatn (mean = 16.0 cm·s^{-1}) compared with char from Lake Øvre Nonshøtjønn (mean = 10.7 cm·s^{-1}) provides further support to the interpretation that these fish may represent, respectively, more pelagic and more littoral–benthic morphs. Although a number of differences between the two populations and their environment (time since stocking, lake elevation, length of growing season, relative size of the pelagic habitat, etc.) may contribute to the appearance of different morphs, the finding of a more pelagic morph in Lake Våvatn and of a more littoral–benthic morph in Lake Øvre Nonshøtjønn is consistent with the presence of brown trout in Lake Våvatn and the scarcity of this species in Lake Øvre Nonshøtjønn (brown trout were stocked in this lake in 1996–1999 but not captured in subsequent gill net surveys). The presence of brown trout has been suggested to promote the shift by char from littoral–benthic to more pelagic habitats (Nilsson 1963; Langeland et al. 1991; Forseth et al. 2003).

In addition to the morphological differences, the char also showed marked between-population differences in their bioenergetics and behavioural attributes. Char from Lake Øvre Nonshøtjønn had a consumption rate 3 times greater and a growth rate 1.9 times greater in the littoral enclosures than char of Lake Våvatn. Char from Lake Øvre Nonshotjønn also fed on prey 2.5 times larger than those ingested by the Våvatn char. Given the similarity in prey communities among the enclosures, particularly at the beginning of the experiment, these results suggest that the char from the two populations differed in prey selection and may have a different quantitative (via consumption rate) and qualitative (via prey size) impact on the structure of the prey community. These results are consistent with the possibility that char from Lake Øvre Nonshotjønn (presumed littoral–benthic morph according to their morphology) may be more efficient at exploiting littoral zone prey than char from Lake Våvatn (possessing a more "pelagic" morphology). The 50% faster mean swimming speed noted for char from Lake Våvatn compared with char from Lake Øvre Nonshotjønn provides further support to the interpretation that these fish may represent, respectively, more pelagic and more littoral–benthic morphs.

Yet, activity rate was similar between char populations and, in contrast with other studies (Marchand and Boisclair 1998), may not have played a role in explaining observed growth differences. Our use of a single swimming cost model for the two morphs studied may have affected our perspective on the role played by activity rate during the experiment. Indeed, the finding that the two morphs differ in morphological attributes that may affect their swimming cost questions the validity of using a single model (Guénard et al. 2008) to estimate swimming cost. However, although we recognize that this approach may be imperfect, estimates of activity rate obtained using video cameras are primarily (87%) driven by the number of fish-seconds observed, with body mass and swimming speed accounting for modest fractions (3.2% and 0.2%, respectively; Trudel and Boisclair 1996; Guénard et al. 2008). It is therefore unlikely that the magnitude of the morphological differences, or potential physiological differences (e.g., metabolic rates), could explain the observed differences in activity rate.

The char from Lake Våvatn were far more active in the epibenthic and pelagic sections of the enclosures relative to char from Lake Øvre Nonshotjønn. Although important differences in mean activity rate could be observed between treatment replicates for char from Lake Øvre Nonshotjønn, these fish nevertheless tended to make more complete use of the enclosures than those of Lake Våvatn. Population-specific strategies for modulating activity among zones and, in particular, the observation that char from Lake Øvre Nonshotjønn may be more inclined to be active in the complete suite of environmental conditions available in the littoral zone are consistent with their presumed identification as the more littoral–benthic morph.

Our results provide additional support to the suggestion that polymorphism may have significant ecological implications not only for life history traits (Skúlason and Smith 1995; Smith and Skúlason 1996; Klemetsen et al. 2003), but also for fitness indices related to bioenergetics (growth and consumption rates) in particular. Such findings may affect the use of trophic cascade models, used to estimate carrying capacity of aquatic ecosystems (Paine 1980; Pace et al. 1999; van Leeuwen et al. 2007), and numerical habitat models, used to predict the impact of physical perturbations on the productive capacity of fish habitats (Boisclair 2001). These models generally assume that fish of a given species and size will perform similarly in specified environmental conditions. In our study, growth and consumption rates differed substantially between morphs maintained under similar environmental conditions. Hence, predictions of growth or consumption obtained using models developed for one morph may not adequately represent the potential performance of another morph under the same environmental conditions. Furthermore, the diet of these morphs may be dominated either by cladocerans or by copepods. These differences in consumption rate and prey selection suggest that predictions of the impact of a given morph on a trophic network may differ quite significantly from predictions based on another morph. The magnitude of the differences in growth and consumption rates and habitat use noted here suggest that a better understanding of the functional implications of polymorphism within a species may improve predictions made by numerical habitat and trophic cascade models.

The experimental design used in the present study is admittedly incomplete. Bioenergetics and behavioural attributes of two morphs of Arctic char from two lakes were estimated and compared only in the littoral zone and only in one lake. A better knowledge of the functional differences between these morphs may be achieved by comparing bioenergetic and behavioural attributes in a more complete suite of habitat types (littoral, pelagic, and profundal) and in other lakes (in the present case, cross experimentation in Lake Våvatn and Lake Øvre Nonshotjønn). This was not done in the present study because of logistical constraints and lack of pre-existing support to justify such an endeavour. However, despite this limitation, we were able to demonstrate functional differences between morphologically distinct morphs of Arctic char that appear to drive important differences in their bioenergetic and behavioural responses when exposed to similar environmental conditions. Given the rather large differences in bioenergetic and behavioural attributes reported here, such functional differences might significantly affect the predictions made by habitat and trophic cascade models involving morphs of Arctic char. We submit that the novel approach and methodology applied here, combined with the improved knowledge of the potential functional differences between morphs, are valuable for future studies on ecological and evolutionary patterns of other polymorphic fish species.

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Appendix A. Video analysis and computations

Video analysis

VHS recordings were converted to numeric format using a computer interface (ATI video acquisition interface, resolution 640 × 480 pixels), and image analysis was performed with software developed by the first author using XBasic Program Development Environment (2002, version 6.2.3). For every second of recording, all moving char (velocity > 1 $\mathrm{cm\cdot s^{-1}}$) that were observed simultaneously in both cameras of a given SVC were counted. For each recording (30 min), a single value of the number of moving fish-seconds (FS, $\mathrm{fish\cdot s^{-1}}$) was obtained by summing the number of fish observed in every second. Positioning of the head and tail of fish (in raw pixel coordinates) was done manually in both images rendered by the SVC (for details, see Boisclair 1992). Swimming speeds of char were estimated on a single 1 s interval, randomly selected over the complete displacement of a given fish in the volume sampled by the SVC.

Computations

Calibration of the SVC

Calibration of the SVC was done underwater using a target consisting of a board with lines spaced 10, 20, and 30 cm apart. This target was placed at distances ranging from 40 cm to 240 cm from the SVC (increments of 40 cm). Calibration was done before the first filming day and was repeated every four filming days. A nonlinear function was used to estimate real lengths (L, cm) from virtual lengths (l, pixels) and parallax (i.e., the offset between images from the cameras in abscissa (Δx), in pixels):

$$\text{(A1)} \qquad L = \frac{l}{\frac{c_1}{\Delta x + c_2} + c_3}$$

where c_1 is a scale parameter, and c_2 and c_3 are offset parameters. Parameters c_1, c_2, and c_3 were estimated by least-squares nonlinear regression of known distances between pairs of landmarks on the calibration target against values of Δx and l obtained from image analysis. The quotient of real and virtual length (L/l) was used to evaluate the distance between the target and the SVC (z, cm):

$$\text{(A2)} \qquad z = c_4 \cdot \frac{L}{l} + c_5$$

where parameters c_4 and c_5 were estimated using linear regression of known values of distance of the calibration target and known values of L/l. Coefficients of determination associated with these relationships (eqs. A1 and A2) ranged from 92% to 99%.

Volume sampled by the SVC

The volume filmed by a video camera has the shape of a rectangle-based pyramid, with the volume increasing with the distance from the camera. This volume crossed the bottom of the littoral zone or the surface of the lake at an angle. Hence, the volume sampled by the SVC was calculated as the volume of a truncated rectangle-based pyramid. This volume began at 25 cm from the objective of the cameras (minimum distance to perceive a fish simultaneously in two cameras of the same SVC) and extended to a maximum distance of fish detection determined using the coordinates of the head and tail of the fish observed during filming. The volumes sampled by the SVC for different recordings were grouped according to filming conditions defined by the zone sampled, the position and the orientation of the cameras, and the characteristics of the images (pixel brightness and their frequency distribution). The volumes sampled by the SVC for recordings during which no fish was observed were used as the average volume sampled by the SVC when fish were observed under similar filming conditions.

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ERRATUM / ERRATUM

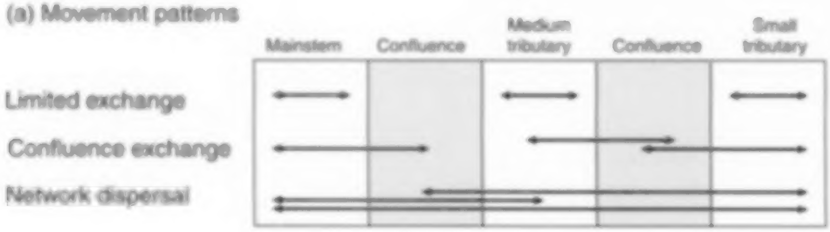
Erratum: Influence of spatial positioning within stream networks on fish assemblage structure in the Kansas River basin, USA

Darren J. Thornbrugh and Keith B. Gido

Ref.: Can. J. Fish. Aquat. Sci. **67**: 143-156 (2010).

Figure 1*a* of this article is a conceptual diagram representing hypothesized movement patterns. The arrows represent species or sizes classes of fish and their potential movements through confluence zones and among streams of different size. In the original article, some of the arrows for confluence exchange and network dispersal are partly missing; the correct diagram for Fig. 1*a* is as follows:

Fig. 1*a*.


(a) Movement patterns
Mainstem
Confluence
Medium tributary
Confluence
Small tributary
Limited exchange
Confluence exchange
Network dispersal

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